SOME EFFECTS OF TRICHOSTRONGYLIDOSIS ON METABOLISM AND PRODUCTION OF SHEEP

by

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in the Faculty of Veterinary Medicine,
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May, 1989.

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ACKNOWLEDGEMENTS

The research for this thesis was undertaken as an AFRC Veterinary Research Fellow, and was supported by an AFRC project grant. This financial support is gratefully acknowledged.

I particularly thank Professor P.H. Holmes and Dr. J.J. Parkins, my supervisors, for their patient guidance, ready assistance and helpful advice.

I am also grateful to Professor J. Armour, Professor W. Mulligan, Dr. J.M. Maclean, Dr. G. Fishwick and Dr. N.S. Ritchie, among other colleagues, for their helpful discussions, and to Mr. T.C. Aitchison for guiding me through the maze that is statistics.

My experience has been enriched by the generous collaboration and cooperation of a range of people, and I particularly thank Dr. B.W. East and Dr. T. Preston for the neutron activation analysis described in Section 3; Dr. D.J. Flint for some of the endocrine assays in the same section; Dr. P.C. Thomas and Mr. S. Robertson for the facilities and assistance with the calorimetric study (Section 5); and Dr. F.A. Harrison for his philosophical counsel.

I am grateful for the friendship and willing assistance afforded me in the Department of Veterinary Physiology and I acknowledge with particular thanks the skilled technical contributions of Kris Cairns, Alan McNair and Raymond Montgomery. I also thank members of the technical staff of the Departments of Veterinary Animal Husbandry, Veterinary Parasitology, Veterinary Pathology and Veterinary Biochemistry for providing patient teaching and conscientious assistance whenever required; staff of the Medical Illustration and Photography Departments; and the stockmen at Garscube and Cochno for

their care of the sheep.

I am indebted to several students who assisted me with skill and enthusiasm, and who have left me with distinctive and memorable recollections of each summer: Deirdre Wilson, Elaine Wilson, Juliet Holmes, John Golding and my sister, Jane Pinkerton.

Above all, I thank God for His guidance and inspiration; my husband Brian for his unfailingly tolerant support; my parents for their patient and practical assistance; and my unborn child for unwittingly encouraging me to to complete this tome. To these, with love, I dedicate this thesis.

SUMMARY

This thesis is concerned with the effects of infection with trichosytrongylid parasites on production and metabolism of sheep, and includes a review of relevant literature and descriptions of a series of experiments conducted to investigate particular aspects of this subject area.

Section 1 comprises a review of previously published work on the subject of trichostrongylidosis with particular reference to the effects of infection with Haemonchus contortus, Trichostrongylus colubriformis and Ostertagia circumcincta in sheep.

In Section 2 are described the materials and analytical techniques used in the experimental work reported in later sections.

Section 3 describes two experiments primarily conducted to measure the effects of two patterns of infection with H. contortus on body composition and feed utilisation in growing lambs offered a high quality diet ad libitum. A limited study was made of endocrinological status and some biochemical parameters were measured in addition to standard parasitological and haematological investigations.

Inoculation of 10-week old parasite-naive lambs with 350 H. contortus larvae/kg BWt resulted in patent infections and the lambs became moderately anaemic. Similar lambs, challenged with small doses (20 larvae/kg BWt) twice weekly, developed a milder anaemia, although the total number of larvae administered was greater. Infection had no effect on feed utilisation or growth and body composition measured over 49 to 109 days, however the single infection caused an alteration in the digestibility of certain

fractions of the diet, six weeks after infection, and nitrogen retention was reduced by both patterns of infection at this time. In other parameters measured the only significant effects of infection were some small changes in endocrine concentrations and an increase in blood glucose concentration. It was suggested that the high level of nutrient intake by infected lambs in this study may have largely eliminated the potential detrimental effects of H. contortus on host metabolism.

This hypothesis was tested in an experiment described in Section 4. Lambs were infected with 350 H. contortus larvae/kg BWt and offered one of two restricted amounts of the same feedstuff used in the previous experiment. Infection resulted in the sheep developing a moderate anaemia as a result of blood loss into the abomasum (confirmed by radioisotopic studies), but there was no apparent effect of infection on feed intake or bodyweight gain within each dietary group. However, infection was found to affect body composition and feed utilisation, and these effects were more pronounced in the lambs maintained on the lower plane of nutrition.

Section 5 describes an investigation of the partitioning of dietary energy in subclinical haemonchosis using indirect calorimetry. Sheep infected with 350 H. contortus/kg BWt were only mildly affected by infection as measured by faecal egg counts and haematological changes. Faecal and urinary energy losses were not significantly affected by infection, nor was there a significant effect on heat production calculated indirectly from gas exchange figures. However, infected sheep lost a greater proportion of dietary energy as methane than did controls, and although this was not reflected in a significant difference in energy retention by the

infected sheep in this experiment, it does suggest that abomasal parasites such as H. contortus may exert an effect on rumen function by an as yet unidentified mechanism.

Three experiments described in Section 6 were conducted to try to explain the low pathogenicity and poor parasite establishment experienced in the previous experiment. A small experiment compared the establishment of the suspect strain of H. contortus with a different strain of known pathogenicity, and appeared to exonerate the original strain. The second measured the potential effect of a sodium sulphate/ammonium molybdate supplement on the establishment and pathogenic effects of the laboratory strain of H. contortus. This supplement had been administered to sheep in some previous experiments to help prevent copper toxicity which had previously been a problem in similar housed lambs. The chemicals did not appear to affect the parasites. The possibility that lambs of the type used in these experiments develop resistance to H. contortus with age was investigated in a third experiment which showed that nine-month old Suffolk x Greyface, and Finn Dorset, sheep were susceptible to challenge with H. contortus and, indeed, were unable to mount an effective immune response to the parasite after vaccination with radio-attenuated larvae. Therefore the reason for the poor performance of the H. contortus in previous experiments remains unresolved.

The final chapter, Section 7, describes the pathophysiological effects of prolonged challenge of mature immune ewes with O. circumcincta larvae. Increased plasma pepsinogen concentrations and losses of plasma into the gastrointestinal tract persisted throughout the 121 day challenge period in two of the three

challenged ewes, although the parasites did not establish in the sheep.

SECTION 1

Some effects of trichostrongylidosis on metabolism and production of sheep: Introduction and literature review.

Section 1

Some effects of trichostrongylidosis on metabolism and production of sheep: Introduction and literature review.

1.1 Introduction

Trichostrongylidosis is the term used for the diseases caused by infection with one or more of the gastrointestinal nematode parasites of the family Trichostrongylidae (Dunn, 1978). Genera of trichostrongylid parasites are common in all the main animal-rearing areas of the world, and present a particular problem in tropical, subtropical and wet temperate regions where conditions are most favourable for the development and survival of infective stages. Members of the family are all parasitic, and exhibit a direct life-cycle with a free-living preparasitic phase and a parasitic phase during which they inhabit a section of the gastrointestinal tract of the host. Ostertagia and Haemonchus spp., and one species of Trichostrongylus, T. axei, inhabit the abomasum or true stomach of ruminants. Other Trichostrongylus spp., and Cooperia and Nematodirus spp. infect the small intestine of ruminants (Soulsby, 1982).

Natural infections with trichostrongylid parasites are rarely single infections; several species may be found in a single host, although one species may dominate numerically and, or, clinically in the production of disease. Much recent experimental work, however, has involved the introduction of a single species into parasite-free animals, and a considerable volume of knowledge has been established on the effects of such infections.

Trichostrongylidosis constitutes an important economic loss to animal-rearing throughout the world. Losses result most obviously from deaths, and also from early culling and reduced production and quality of milk, meat and wool from infected animals. Reproductive performance may be impaired and genetic selection will be reduced as a consequence of this and deaths resulting from the disease. The cost of veterinary care and anthelmintics must also be included. It has been estimated that, if left untreated, the economic impact of trichostrongylidosis in cattle and sheep would approach £130 million per annum in the U.K. alone (Professor G.M. Urquhart, personal communication).

This review is concerned with the various effects of trichostrongylidosis on animal production, and the alterations in gastrointestinal function and host metabolism which accompany trichostrongylid infections. It is largely restricted to considerations of the effects of three ovine parasites, H. contortus, O. circumcincta and T. colubriformis, but references to other parasites are included where appropriate. Firstly it seems pertinent to outline briefly the life-cycle of these parasites, and the principal clinical and pathological features of the diseases they cause, which throughout this thesis are termed haemonchosis, ostertagiasis and trichostrongylosis respectively.

1.1.1 Life-cycle of the Trichostrongylidae

All species of Trichostrongylidae exhibit a direct life-cycle comprising two phases, preparasitic and parasitic.

In the preparasitic phase, eggs passed in the faeces of an infected animal hatch, under suitable environmental conditions, into motile first-stage larvae, L_1. These remain in the faecal pat and

ingest small particles of faecal matter. The L_1 moult into L_2 which continue to feed and develop, and then undergo a second moult or ecdysis to become L_3. These third-stage larvae do not cast the sheath of the L_2, and remain inside it, unable to feed but protected from adverse environmental conditions. The sheathed L_3 are the infective stage. Once ingested by a suitable host they exsheath and inhabit their predilection site within the gastrointestinal (GI) tract. There is no visceral or vascular migration. The L_3 moult twice more either within or in apposition to the mucosa to become L_5 which then develop without further ecdysis to the adult stage. Mating takes place, and the life-cycle is completed when the female produces eggs which are carried away by the gut contents and excreted in the faeces.

1.1.2 Pathological and clinical features of ovine haemonchosis

Following infection with H. contortus, early development of larvae in the abomasum occurs in the gastric glands, in the lamina propria and at the mucus/mucosal junction and is accompanied by a degree of mucosal hypertrophy and the presence of excess mucus (Charleston, 1965; Malczewski, 1970; Hunter and MacKenzie, 1982). The most notable pathological feature is traumatic damage to the abomasal mucosa which results from the haematophagic activities of L_5, adult and, to a lesser extent, fourth stage larvae. This is evident grossly as pinpoint haemorrhages on the mucosal surface, and histologically as small surface erosions with loss of epithelial cells and obvious capillary haemorrhage (Hunter and MacKenzie, 1982).

From the time of larval development to the late L_4 stage, there is a fairly constant GI loss of red cells until such time as the adult parasites are expelled (Dargie and Allonby, 1975). Each

worm removes about 0.05 ml blood per day (Clark, Kiesel and Goby, 1962) and the major clinical feature accompanying infection is a haemorrhagic anaemia (Fourie, 1931; Campbell and Gardiner, 1960; Abbott, 1982). From the work of Dargie and Allonby (1975) and of Abbott (1982), it is apparent that the anaemia of haemonchosis shows three stages of development. The first stage occurs during the initial three weeks of infection, and is characterised by a progressive fall in the packed cell volume (PCV), often to around 70% of the pre-infection value. Normal serum iron concentrations, and normal red cell volumes (MCV) and mean cell haemoglobin concentrations (MCHC) are features, and the anaemia is attributed to a latency in the response of the host's erythropoietic system. The second stage, which may not involve further reduction in PCV, is nonetheless accompanied by continued abomasal haemorrhage and marked stimulation of erythropoiesis achieved at the expense of the animal's iron stores. This second stage anaemia is normochromic and macrocytic. The final stage, observed in animals of low nutritional status, is characterised by a dramatic reduction in PCV, MCV, MCHC and serum iron concentration and represents failure of the host's synthetic machinery due to exhaustion of reserves of iron and, possibly, available amino acids.

Allonby (1973) and Allonby and Urquhart (1975) described three clinical syndromes of haemonchosis in Kenya.

Hyperacute haemonchosis was rare and of sporadic occurence. It was characterised by sudden death from severe haemorrhagic gastritis and severe anaemia following a sudden massive challenge of infective larvae in a period of warm, humid weather.

In animals subjected to less massive challenge, the classical

signs of acute haemonchosis developed. These include anaemia, generalised oedema and 'bottle-jaw' or submandibular oedema, loss of condition, and weakness in terminal cases.

The authors also described the previously unrecognised 'chronic haemonchosis' syndrome, in which moderate anaemia and progressive weight loss and ill-thrift developed in sheep carrying low worm burdens and subjected to poor nutrition in the dry season.

1.1.3 Pathological and clinical features of ovine ostertagiasis

In ostertagiasis, exsheathed larvae penetrate the gastric glands of the abomasal mucosa where they moult twice before emerging as young adults. As the larvae develop, the glands become stretched, their epithelium becomes hyperplastic and the secretory cells are replaced by undifferentiated, non-secretory cells. These changes take place not only in the glands occupied by developing larvae, but also in those surrounding them (Armour, Jarrett and Jennings, 1966). Where infection is severe the result is a thickened, undifferentiated epithelium, lacking secretory function and also the physical function of preventing leakage of fluid and large molecules inward and outward between the cells. The effects of this are an increased abomasal pH, increased concentration of plasma pepsinogen (Armour et al, 1966), and a loss of plasma proteins into the gastrointestinal tract (Holmes and Maclean, 1971).

Two clinical syndromes are recognised in Britain, which correspond epidemiologically to the two forms of bovine ostertagiasis described by Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965). Type I ovine ostertagiasis, which occurs in young lambs in July to September, is characterised by sudden onset profuse diarrhoea accompanied by dullness and lethargy followed by weight

loss or reduced weight gain in surviving lambs. Type II disease is associated with emergence from the gastric glands of inhibited larvae in late winter and early spring. Affected sheep are usually six months to two years old, and they show progressive weight loss with intermittent soft faeces or diarrhoea (Reid and Murray, 1973).

1.1.4 Pathological and clinical features of ovine trichostrongylosis

Intestinal Trichostrongylus spp. develop within sinusoidal tunnels in the epithelium of the proximal third of the small intestine and the associated mucosa shows stunting or atrophy of villi with sparse, irregular microvilli on domed enterocytes (Barker, 1973a; 1975a; Coop and Angus, 1975). Emergence of the worms from ruptured worm tunnels at around 15 days after infection is accompanied by an inflammatory response (Taylor and Pearson, 1979a; b). Macroscopically, focal 'finger-print' lesions, 1 to 2 cm in diameter may be evident. These are flattened, bright red depressions, sharply demarcated from surrounding normal beige-coloured mucosa. In severe cases these lesions become confluent, particularly in the first 1.5 m of the intestine (Coop, Angus and Sykes, 1979).

There is an increased plasma loss into the gut (Barker, 1973b) and inappetance, hypoproteinaemia and weight loss are evident in affected animals. Diarrhoea is an occasional feature.

Infection with T. vitrinus produces lesions and clinical effects which are similar to, though less severe than, those which result from infection with comparable numbers of T. colubriformis (Coop, Sykes and Angus, 1976; Coop et al, 1979).

1.2 Effects of trichostrongylidosis on animal production

Barger (1982) considered that 'perhaps the only generalisation that could be made about the effects of helminth parasites on animal production is that production is not increased by helminth parasitism.' Whether or not trichostrongylid parasites will reduce production in a given situation depends on the number and species of parasites involved, and on the age, breed, immunological and nutritional status of the host animal. These in turn are influenced by a combination of environmental factors and management practices. However, as this review will show, trichostrongylidosis has been demonstrated to reduce animal production in various circumstances, through increased mortality, reduced growth rate, altered body composition and decreases in wool production, milk yield and reproductive efficiency.

1.2.1 Mortality

Reports of fatal trichostrongylid infections are widespread in the literature, and a selection of such references are presented here.

Scott, Silverman, Mansfield and Levine (1971) and Abbott (1982) reported deaths among lambs given experimental infections with H. contortus. Deaths were recorded among sheep experimentally infected with O. circumcincta by Symons, Steel and Jones (1981) and with T. colubriformis by Prichard, Hennessy and Griffiths (1974) and Sykes, Coop and Angus (1975).

Fatalities among sheep naturally infected with H. contortus have been widely reported, eg. in Kenya by Allonby (1973) and in Nigeria by Eysker and Ogunsusi (1980).

Studies have demonstrated that anthelmintic treatment reduces

mortality among animals exposed to a field infection with H. contortus (Banks, Hall and Korthals, 1966) or to a mixed trichostrongylid infection (Anderson, Morris and McTaggart, 1976).

1.2.2 Bodyweight changes

Loss of bodyweight, or a reduction in growth rate, is regularly reported in animals infected with trichostrongylid parasites and is the most common criterion for judging the effects of such parasites on animal production.

In haemonchosis, Dargie (1973) reported a reduction in growth rate of 26% in infected lambs relative to pair-fed controls. Abbott (1982) found that animals infected with H. contortus tended to grow less well than uninfected controls, the difference varying with the age, breed and nutritional status of the sheep, and with the level of parasitic challenge. Other reports of weight loss or impaired weight gain in sheep infected with H. contortus include those by Kates, Allen and Wilson (1962), Malczewski (1970), Silverman, Mansfield and Scott (1970), Allonby and Urquhart (1975) and Bezubik, Stankiewicz and Byszewska-Szpocinska (1978).

Considering ostertagiasis, Sykes and Coop (1977) reported that growth rates of lambs given 4,000 O. circumcincta per day were 80 and 60% of the rates achieved by pair-fed and ad libitum controls respectively. In a subsequent experiment (Coop, Sykes and Angus, 1982), growth rates of lambs receiving 3,000 - 5,000 larvae per day ranged from 75 to 53% of that of the ad libitum control group. Symons et al (1981) and Steel, Jones and Symons (1982) found that challenges of 37,500 to 120,000 O. circumcincta larvae per week reduced liveweight gains relative to lambs receiving fewer, or no, larvae.

More work has been conducted concerning the effect of infection with T. colubriformis on growth rate. Barker (1973b) found that infection with 85,000 T. colubriformis larvae reduced growth rate to 34% of that of uninfected control lambs. Reveron, Topps, MacDonald and Pratt (1974b) reported that of lambs infected with 40,000 T. colubriformis, those considered to be slightly affected and those severely affected achieved growth rates of 70 and 21% respectively of the growth rate of uninfected controls.

Coop et al (1976), comparing lambs given 2,500 T. colubriformis per day with pair-fed and ad libitum control groups, found that infection suppressed growth to 58 and 46% of that of the respective control groups.

Liveweight gain of lambs given 3,000, 9,500 and 30,000 T. colubriformis larvae per week were, respectively, 67, 61 and 49% of the gain of uninfected lambs (Steel, Symons and Jones, 1980). Subsequently, Jones and Symons (1982) reported that lambs given 18,000 T. colubriformis larvae per week lost 0.02 kg bodyweight per week, in contrast to gains of 0.38 and 0.68 kg per week by uninfected pair-fed and ad-lib controls respectively.

MacRae, Smith, Sharman, Corrigall and Coop (1982) reported that lambs infected with 2,500 T. colubriformis larvae per day grew as well as pair-fed controls for the first 3 to 4 weeks, but thereafter the infected lambs showed little weight gain whereas pair-fed controls continued to gain weight.

Considering mixed infections, Steel et al (1982) reported that concurrent infection with 3,000 T. colubriformis and 38,000 O. circumcincta larvae per week reduced weight gain by 15 kg over 16 weeks, compared to uninfected controls. The same dose of

O. circumcincta given alone reduced weight gain by only 6 kg, whilst the T. colubriformis component given alone produced no suppression of growth.

Anderson et al (1976) found that fortnightly anthelmintic treatment of lambs grazing infected pasture improved average weight gain by 5 kg over untreated, or less frequently treated, lambs.

1.2.3 Body composition

It has been known for some time that sheep suffering from trichostrongylidosis tend to be unthrifty, lose body condition and, in severe cases, become emaciated (eg. Gallagher, 1962; Allonby and Urquhart, 1975; Eysker and Ogunsusi, 1980).

More recently, attempts have been made to quantify the effects of trichostrongylid infections on body composition.

Experiments have shown that carcases of sheep infected with T. colubriformis contain less protein and have a lower energy content than carcases of uninfected pair-fed controls. This is accompanied by an increase in water concentration in the fat-free empty body (Sykes and Coop, 1976). In addition, sheep infected with this parasite show reduced deposition of Ca and P in the skeleton (Reveron, Topps and Gelman, 1974a; Sykes and Coop, 1976) and osteoporosis develops (Sykes et al, 1975). The weight of the dressed carcase as a percentage of liveweight (killing-out percentage) may be reduced (Steel et al, 1980).

Sykes and Coop (1977) found similar effects with O. circumcincta infection, and the same authors showed that the magnitude of the effects were related to the level of parasitic challenge (Coop, Sykes, Spence and Aitchison, 1981; Coop et al, 1982).

It is interesting to note that in none of these experiments did any of the sheep exhibit clinical signs of parasitism, with the exception of a moderate degree of anorexia.

Abbott (1982) conducted a limited study of carcase composition in sheep infected with H. contortus by dissecting an indicator joint, the 7th to 10th ribs. She found a reduced percentage of muscle in infected sheep relative to controls, the difference being most marked among sheep fed a low protein diet.

1.2.4 Wool production

It is well established that trichostrongylidosis can be accompanied by a reduction in the quantity and quality of wool produced. Carter, Franklyn and Gordon (1946) reported that sheep infected with T. colubriformis produced 60% less wool than uninfected controls.

More recently, the relationship between wool production and the level of parasite challenge has been studied. Steel et al (1980) reported that the threshold level of T. colubriformis infection to cause impairment of wool production was between 950 and 3,000 larvae per week. In animals given 30,000 larvae per week wool growth was reduced to 44% of the rate of uninfected control sheep, and wool fibre diameter was significantly reduced. In the case of infection with O. circumcincta, 1,200 or more larvae per week reduced wool production significantly (Symons et al, 1981). Lambs infected concurrently with T. colubriformis and O. circumcincta experienced reduced wool production of up to 66% (Steel et al, 1982).

Total wool growth, wool fibre diameter and staple strength were all reduced in ewes infected with O. circumcincta during the first

six weeks of lactation (Leyva, Henderson and Sykes, 1982).

Wool production can be affected by trichostrongylid infection even when the sheep appear otherwise resistant to infection. Wool production was impaired by 11 to 18% when sheep resistant to T. colubriformis were exposed to larval challenge (Barger, Southcott and Williams, 1973; Barger and Southcott, 1975).

The above-mentioned studies described reduced wool production in animals given an experimental infection relative to uninfected controls. In investigations of anthelmintic efficacy, many workers have reported improved wool production as a benefit of such therapy. Banks et al (1966) found that anthelmintic-treated lambs produced twice as much wool as untreated lambs grazing pasture contaminated with a mixed trichostrongylid infection. Similarly, Johnstone (1978) reported that wool production was reduced in weaner, yearling and two-year-old Merino wethers infected with H. contortus when compared to anthelmintic-treated controls.

1.2.5 Milk production

The few studies that have measured the effect of trichostrongylid challenge on milk production in ewes have shown that infected animals may suffer an impairment in milk yield.

Gordon (1950) reported that milk production by a single ewe declined when the ewe was infected with H. contortus and subsequently increased after anthelmintic treatment. Ewes infected with H. contortus over the last six weeks of pregnancy and first six weeks of lactation showed a marked weight loss during lactation and gave 23% less milk compared to uninfected controls on the same level of feed intake (Thomas and Ali, 1983).

Leyva, Henderson and Sykes (1981) and Leyva et al (1982)

challenged ewes with O. circumcincta daily for the first six weeks of lactation. Milk production in these infected ewes fell more rapidly from peak values and by the sixth week of lactation was significantly lower than that of control sheep.

1.2.6 Reproduction

Since trichostrongylidosis can affect all other aspects of animal production, it seems likely that reproductive performance may be adversely affected by parasitism although there are as yet few data on this subject.

From analysis of 81 field trials, Lewis (1975) found that ewes given a single anthelmintic drench during the six weeks prior to the start of mating produced 3.6 % more lambs. This was due to increased twinning, as barrenness was not reduced. The effect of treatment was most marked in flocks with a mean faecal egg count above 100 eggs per g before treatment, and where the treated ewes showed a live-weight increase.

Johnstone, Coote and Smart (1979) found that pre-lambing anthelmintic treatment of grazing pregnant ewes increased lamb birth weights.

Mackay (1980) studied the effect of different worming routines on the performance of Scottish hill flocks. He found that barrenness was reduced and rearing percentages were increased in flocks treated between mating and parturition.

1.3 Effects of trichostrongylidosis on gastrointestinal function

1.3.1 Feed intake

Anorexia, or a reduction in voluntary feed intake, is common among animals infected with most of the common trichostrongylids and is responsible for much of the reduced productivity observed in such animals. Intake may be reduced by more than 20% in severe cases of trichostrongylidosis (eg. Reveron et al, 1974b), and the degree of anorexia is usually related to the level of parasite challenge (Steel et al, 1980; Coop et al, 1982).

It is possible to measure the extent to which reduced production is attributable to anorexia by conducting experiments using three groups of animals. The first group are infected and fed to appetite. Animals of one control group are pair-fed to individual infected animals, whilst the other uninfected group is offered feed ad libitum. The total effect of parasitism on production can be measured by comparing the infected group with the ad libitum control group. The extent to which anorexia alone is responsible for reducing production is the difference between the pair-fed and ad libitum control groups.

Using this method Sykes and Coop (1976) found that a reduction in food intake of 16% in trichostrongylosis accounted for 37% of the reduced weight gain observed in the infected sheep. In ostertagiasis infected sheep ate 20% less food and this accounted for 38% of the reduction in weight gain (Sykes and Coop, 1977).

In the case of haemonchosis anorexia is a more variable feature. Evans, Blunt and Southcott (1963), Kates et al (1962) and Pradhan and Johnstone (1972) all reported reductions in feed intake by sheep infected with H. contortus whereas other workers, including

Southcott, Heath and Langlands (1967) and Dargie (1973) found that infected sheep tended to eat as much as, or more than, uninfected controls. Abbott (1982) demonstrated that the level of protein nutrition can affect feed intake in haemonchosis - infected sheep maintained on a low protein diet became anorexic whilst those offered a high protein diet maintained appetite.

Much emphasis has been placed on anorexia in parasitic infections, but Sykes (1982) pointed out that parasitised animals tend to undergo less skeletal growth than controls, and thus have a smaller bodyweight to maintain. He recalculated feed intakes from a number of experiments on a unit bodyweight basis, and showed that in chronic parasitism infected sheep may eat more food per unit bodyweight than their larger controls.

The mechanisms underlying anorexia in trichostrongylidosis remain unclear. Several authors have proposed that abdominal pain may be the cause, or part of the cause, of anorexia (Andrews, 1939; Gibson, 1955) and in severely affected sheep clinical signs such as teeth grinding and depression, which are generally associated with abdominal pain, have occasionally been observed. However these signs are not common, and the true role of subjective factors such as pain is difficult to assess.

Trichostrongylid infections are associated with local pathological changes, as discussed above, and many workers have implicated these lesions as the cause of the anorexia (eg. Reveron et al, 1974b). This theory seems plausible, since the appetite centres are situated in the hypothalamus (Morley, 1980), and inflammation of the gastrointestinal tract has been shown to interfere with autonomic reflexes and neurological input to the

hypothalamus (Hall, 1975).

Berry and Dargie (1976) noted that in ovine fascioliasis both feed intake and clinical condition tended to decline with increasing duration of infection, and more rapidly when the host was offered a poor quality diet. From this Dargie (1980) concluded that it is 'the clinical condition of the animal as reflected in its haematological and/or plasma protein levels which somehow also determines the level of feed intake.' Whilst appetite and clinical condition are likely to be related, there is no good evidence to suggest which is the causal factor and which the effect.

Alterations in GI secretions and permeability, the rate of passage of ingesta, motility of the GI tract and levels of gut hormones have all been recorded in parasitised sheep and implicated in anorexia, and a discussion of these findings is included below. However, whether any of these factors cause, or alternatively result from, anorexia remains unproven.

1.3.2 Gastrointestinal secretions

Abomasal infections with H. contortus and O. circumcincta have been shown to cause changes in abomasal secretory behaviour resulting in similar effects on abomasal pH and mineral content.

Infection with H. contortus induces an increase in abomasal bicarbonate concentration within 30 minutes of intraabomasal administration of infective larvae (Bueno, Honde, Luffau and Fioramonti, 1982b), a decrease in K^+ and Cl^- concentrations from day 3, and a pH increase from the fourth day post-infection (Christie, Brambell and Mapes, 1967; Coop, 1971; Dakkak, Bueno and Fioramonti, 1981).

Similarly, infection with O. circumcincta induces a decrease

in K^+ and Cl^- concentrations (Armour et al, 1966) and an increase in abomasal pH from 8 days post-infection (Armour et al, 1966; McLeay, Anderson, Bingley and Titchen, 1973; Sykes and Coop, 1977).

These changes are all indicative of a decrease in abomasal secretion of hydrochloric acid which, in ostertagiasis at least, has been associated with a decline in the number of acid-secreting parietal cells in the gastric glands (Armour et al, 1966; Murray, Jennings and Armour, 1970).

In a study using sheep surgically prepared with fundic pouches, McLeay et al (1973) showed that while acid secretion was reduced in the parasitised 'abomasum proper', the output of acid and pepsin from uninfected pouches was actually increased in sheep infected with O. circumcincta.

Few studies have measured secretions in intestinal trichostrongylidosis. Abomasal pH has been shown to rise after infection with T. colubriformis. Parietal cells were less prominent and showed ultrastructural changes, and, in contrast to ostertagiasis, acid secretion from parasite-free fundic pouches was reduced (Barker and Titchen, 1982).

Work has shown that the mucosal activities of certain intestinal enzymes are significantly altered in lambs chronically infected with T. colubriformis (Symons and Jones, 1970; Jones, 1983) or T. vitrinus (Jones, 1982).

1.3.3 Gastrointestinal permeability

It is now well established that the permeability of the GI tract is altered in trichostrongylidosis.

Losses of plasma proteins into the GI tract have been

demonstrated using radioisotopic techniques, and these will be discussed more fully below.

An increase in the plasma concentration of pepsinogen occurs in ostertagiasis (Armour et al, 1966; Holmes and Maclean, 1971; McLeay et al, 1973; Sykes and Coop, 1977) and, to a lesser extent, in haemonchosis (Coop, 1971; Kerboeuf, 1977; Dakkak et al, 1981).

An increase in the concentration of Na^+ in gastric or abomasal contents suggests a loss of interstitial fluid (Davenport, 1964), and such a change has been observed in sheep infected with H. contortus (Coop, 1971; Dakkak et al, 1981) and with O. circumcincta (Armour et al, 1966; McLeay et al, 1973; Sykes and Coop, 1977).

Changes in abomasal permeability in ostertagiasis are normally associated with the emergence of mature larvae from gastric glands of susceptible sheep (Holmes and Maclean, 1971). However studies have shown that significant increases in plasma pepsinogen (Anderson, 1973; Yakoob, Holmes and Armour, 1983) and GI plasma loss (Yakoob et al, 1983) can occur in the absence of larval emergence when apparently immune adult sheep are exposed to O. circumcincta challenge.

In bovine ostertagiasis, it has been suggested that the alterations in gastrointestinal permeability reflect disruption of tight junctional complexes between mucosal epithelial cells (Murray et al, 1970). In ovine trichostrongylosis, Barker (1975b) used a colloidal carbon labelling technique to demonstrate that in sheep infected with T. colubriformis there is abnormal leakage from capillaries and venules in infected areas of intestinal mucosa. On the basis of this and histological evidence, he deduced that the changes were probably mainly as a result of development of transient

or discontinuous gaps in endothelial cell junctions.

1.3.4 Gastrointestinal motility and digesta flow

Although infection with many of the trichostrongylid parasites can result in diarrhoea, little work has been reported concerning the effects of these parasites on GI motility and digesta flow.

Roseby (1977) measured faecal excretion of ^{51}Cr-EDTA and reported that subclinical infection of lambs with T. colubriformis was associated with a decreased volume of digesta in the rumen, increased volume in the abomasum and intestines, and a slowing of transit through the small intestine.

Bueno, Dakkak and Fioramonti (1982a) described gastro-duodenal motor and transit disturbances in sheep infected with H. contortus. Duodenal flow was increased by nearly 50%, with maximal flows coinciding with the highest frequency of duodenal migrating myoelectrical complexes and the highest abomasal pH and Cl^- concentration. They concluded that the duodenal motor disturbances observed were related to alterations in abomasal acid secretion, and that the increased duodenal flow was a consequence of altered permeability of the GI mucosa.

Wilson and Field (1983) showed that O. circumcincta infection produced a marked increase in the flow of digesta from the abomasum, the extra digesta being reabsorbed in the small intestine.

1.3.5 Gut hormones

There is growing evidence that the host's response to trichostrongylid parasites may be mediated by the action of certain of the gut hormones.

Reynolds, Anderson, Stiffe, Hansky and Titchen (1979) observed hypergastrinaemia in sheep infected with O. circumcincta. At

post-mortem, the abomasal mucosa showed increased gastrin activity, while that of the duodenum was depressed. The authors deduced that there must be an increased rate of production of gastrin, as well as increased circulating concentrations, in this infection.

Hypergastrinaemia was also reported in ovine ostertagiasis by Anderson, Hansky and Titchen (1981) and they proposed that this is a response to the increase in abomasal pH. However, elevations in plasma gastrin have been detected within 24 hours of direct transfer of O. circumcincta adults into the abomasum of previously uninfected sheep, several days before the abomasal pH altered (Titchen, 1982) and this suggests that parasites or their secretions may have a direct effect on gastrin secretion or activity.

In contrast to ostertagiasis, infection with T. colubriformis, which also reduces gastric secretion, resulted in plasma gastrin levels being depressed (Titchen, 1982).

Levels of cholecystokinin (CCK) were elevated in lambs infected with T. colubriformis (Symons and Hennessy, 1981). In the same paper the authors demonstrated that injection of CCK suppressed feed intake in hungry, uninfected sheep, and they postulated a role for this hormone in anorexia in parasitised sheep.

1.3.6 Digestion and absorption

Some early workers claimed that the digestibility of the diet, and crude protein (CP) in particular, was reduced by parasitism (Stewart, 1932/33; Spedding, 1954; Shumard, Bolin and Eveleth, 1957; Horak and Clark, 1964; Owen, 1973). However their conclusions must be viewed with the reservation that they did not take into account the effect of reduced intake on the digestibility of the ration. It is well established that the level of feeding

affects the digestibility of a feedstuff (A.R.C., 1980), and thus in order to measure meaningfully the effect of trichostrongylidosis on digestibility, it is essential to conduct comparative studies on uninfected control animals at the same level of feed intake as the infected group. These uninfected animals are termed pair-fed controls.

Experimental evidence of the influence of haemonchosis on digestibility is sparse and conflicting. Dargie (1980) reported a decrease in the digestibility of CP in lambs 5 - 6 weeks after infection with H. contortus compared to pair-fed controls. However, Abbott (1982) found little evidence from a series of digestibility studies to suggest that infection with H. contortus, at any of the challenge levels selected, impaired the digestion and absorption of any component of the ration.

The data from studies of O. circumcincta are also conflicting. Parkins, Holmes and Bremner (1973) noted a reduction in 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 was observed by Sykes and Coop (1977).

In the case of trichostrongylosis, Andrews, Kauffman and Davis (1944), Roseby (1973) and Sykes and Coop (1976) all presented data which suggests that T. colubriformis infection does not affect the digestibility of the diet. However there is evidence that rumen fermentation may be affected in this infection. In sheep infected with T. colubriformis, Steel (1972) found that acetate production rate was reduced by 76% and 30% relative to ad libitum and pair-fed

controls.

Conventional digestibility trials, such as those reported above, measure the intake and faecal excretion of certain dietary components and calculate that proportion which is not excreted and is thus assumed to be absorbed. It is however an over-simplification to assume that all the faecal material represents undigested food, and that the proportion of food not excreted in the faeces is equal to the total amount which is absorbed from the digestive tract. When considering energy digestibility, it must be remembered that a proportion of dietary energy is lost as eructated methane. In the case of nitrogen digestibility, the presence of metabolic faecal nitrogen in faeces, from sources such as sloughed epithelial cells, mucus, enzymes and, in the case of GI parasitism, blood proteins, results in an under-estimation of the amount of nitrogen absorbed by the animal. Conventional digestibility studies therefore measure the 'apparent' digestibility of a ration, rather than the 'true' digestibility of the feedstuff. As mentioned previously, trichostrongylid infections are associated with an increased loss of endogenous protein into the GI tract. Thus the observation of a reduced protein digestibility coefficient in an infected animal does not necessarily represent a failure to digest or absorb dietary nitrogen. Rather, the increased faecal nitrogen sometimes reported in these infections probably represents a small proportion of the leaked endogenous protein which has escaped digestion and, or, reabsorption. This will be discussed more fully below.

1.4 Metabolic effects of trichostrongylidosis

1.4.1 Protein metabolism

A consistent feature of trichostrongylidosis is loss of endogenous protein into the GI tract. The proteins represent plasma (and in some infections blood cells), exfoliated epithelial cells and mucus.

Much work has been conducted to measure the losses of blood proteins in trichostrongylidosis, mostly using radioisotopic techniques. Losses of plasma can be quantified by measuring the excretion of radioactivity following intravenous administration of ^{125}I-labelled albumin or $^{51}CrCl_3$. Similarly, red blood cell losses can be measured using ^{51}Cr-labelled erythrocytes.

In ovine ostertagiasis (Holmes & McLean, 1971), losses of plasma into the GI tract two weeks after infection were 92 ml/day in infected sheep compared to 29 ml/day in controls. This was associated with hypoalbuminaemia, and an increase in the fraction of the intravascular pool of albumin catabolised per day in infected sheep. Symons et al (1981) reported similar findings in lambs infected with O. circumcincta.

Barker (1973b) reported GI plasma losses in sheep infected with T. colubriformis. In one experiment, twenty-week old Merino lambs which showed signs of trichostrongylosis had elevated GI plasma losses. In a second experiment, however, twelve-week old meat-type crossbred lambs given the same larval challenge showed neither clinical signs nor increased plasma losses despite carrying heavier worm burdens. Nine-week old Merino lambs in a third experiment had increased plasma loss beginning 10 - 12 days after infection and coinciding with the onset of inappetance, hypoproteinaemia and weight

loss.

Steel et al (1980) also recorded increased enteric loss of plasma in sheep infected with T. colubriformis, the extent of the increase being more pronounced at higher levels of larval challenge. Initially this was associated with increased turnover and irreversible loss of albumin, but after hypoalbuminaemia developed the rate of irreversible albumin loss was reduced.

In the case of haemonchosis, Dargie (1973) demonstrated GI losses of plasma in infected sheep of 273 ml/day compared to 38 ml/day in controls. The work of Abbott (1982) revealed a similar picture, and confirmed that sheep infected with H. contortus show an increased fractional catabolic rate and a decrease in the apparent half-life (T 1/2) of albumin.

In haemonchosis, in addition to alterations in plasma protein metabolism, loss of erythrocytes into the gut results in the development of anaemia. Dargie (1973) reported that the sheep mentioned above which lost 273 ml plasma/day also had losses of red cells corresponding to 130 ml of whole blood per day. The loss of whole blood results from the haematophagic activities of the parasites, and the mucosal bleeding which persists for 6 to 7 minutes after cessation of feeding (Boughton and Hardy, 1935, cited by Andrews, 1942). Since more than twice as much plasma was lost as would be explained by the whole blood loss in these sheep, an additional mechanism may exist whereby plasma proteins, but not erythrocytes, escape into the gut. This mechanism may show similarities to the abomasal 'leak' lesion of ostertagiasis, or to the alterations in vascular permeability observed in trichostrongylosis (see above).

In addition to blood proteins, there is some evidence that sloughed cells and mucus contribute to the endogenous protein loss into the GI tract in trichostrongylidosis.

In both T. colubriformis and T. vitrinus infections of lambs the elongated crypts contain excess mitotic figures, an indication of increased cell proliferation and, presumably, cell turnover (Coop and Angus, 1975; Coop et al, 1979). An indication of increased cell turnover in haemonchosis is the finding that ^{3}H-thymidine uptake by abomasal cells is increased in sheep infected with H. contortus (Rowe, Abbott, Dargie and Holmes, 1982).

There are no reports of quantitative measurements of mucus production. However, proliferation of goblet cells at the site of infection has been observed in ostertagiasis (Armour et al, 1966; Murray et al, 1970). Goblet cell hyperplasia has also been reported in lambs infected with T. colubriformis (Coop and Angus, 1975) or T. vitrinus (Coop et al, 1979; Jackson, Angus and Coop, 1983).

There have been several attempts to quantify nitrogen (N) losses into the GI tract, and to determine their subsequent fate, using animals surgically prepared with indwelling cannulae.

Steel (1974, 1978) demonstrated that the partitioning of nutrient uptake between the stomach, small and large intestines may be substantially altered following infection with T. colubriformis. Non-ammonia nitrogen (NAN) flow from the ileum was increased in infected sheep, especially in those maintaining appetite. Faecal N showed a similar relationship with feed intake. Thus the sheep with the greater N losses were, paradoxically, those which were judged to be less severely affected, on the conventional basis of feed intake,

than their inappetant counterparts (Steel, 1974).

In similar studies in sheep infected with O. circumcincta (Steel, 1978), the pattern of NAN movement through the GI tract was also markedly affected. Compared to worm-free controls on similar intakes, infection increased NAN flow from the abomasum and ileum and N output in faeces by a mean of 5.1, 1.1 and 0.5 g/day respectively.

Steel (1978) concluded that in ostertagiasis the majority of the endogenous NAN lost into the abomasum is reabsorbed during passage through the small intestine. However, in trichostrongylosis incomplete reabsorption of endogenous N from the small intestine occurs at higher N intakes, probably when the capacity for absorption is exceeded. He suggested that in concurrent infections with these two parasites the presence of T. colubriformis in the intestine may prevent reabsorption of the N lost in the abomasum infected with O. circumcincta, and the total effect on N absorption might thus be much greater than if either parasite were present alone. In support of this, Steel et al (1982) demonstrated that the production effects of such a combined infection were more severe than would have been expected from the sum of effects of the individual infections.

Poppi, MacRae and Corrigall (1981) used sheep prepared with simple cannulae into the rumen, duodenum and ileum to measure N flow and digestion in trichostrongylosis. There were no differences in the true digestibility of ^{35}S-labelled bacteria in the small intestine or apparent N digestibility over the whole tract, and this confirmed earlier results of Symons and Jones (1970). However the infected lambs of Poppi et al (1981) did show increased loss of plasma protein into the small intestine, increased ileal N flow rate,

and increased urinary N excretion, although the latter difference was not statistically significant.

If their assumption that the true digestibility of bacterial protein (0.709) holds true for all protein entering the small intestine, then the extra 1.5 g ileal N would represent an increase of about 5 g of endogenous protein entering the small intestine. Since less than 1 g of this was plasma protein leakage, they deduced that more than 4 g N must have come from other sources, such as cell sloughing and mucin secretions. However, there is the possibility that mucin, with its protective role in the GI tract, may be less digestible than other proteins, in which case much less than 4 g extra would have to be secreted to account for their observations.

Rowe et al (1982) examined the GI flow of N in cannulated sheep infected with H. contortus, and in similar sheep 'sham' parasitised by infusing appropriate amounts of blood (200 ml/day) into the abomasum to simulate the effect of H. contortus infection. In contrast to the situation in trichostrongylosis described above, they found that blood loss accounted for virtually all of the extra 6 g N/day flowing from the abomasum, and it was all reabsorbed before the terminal ileum.

These studies did not, however, investigate the form in which the N was reabsorbed, nor its fate after absorption from the GI tract. While much of it, particularly in abomasal infections, is probably digested and absorbed as amino acids in the small intestine, there is evidence that not all the N is dealt with in this way.

Roseby (1977) demonstrated an increase in ammonia and VFA concentrations and pool sizes in the caecum-proximal colon of sheep infected with T. colubriformis. From this he deduced that there

was more microbial fermentation than normal in this region, due to increased flow of fermentable substrate from the small intestine. Much of the caecal ammonia would result from microbial deamination of amino acids, so the net effect to the host would be a decrease in amino acid absorption, and increased absorption of ammonia. A higher concentration of ammonia in blood reaching the liver would result, and this would explain the higher rate of urea synthesis recorded by Roseby and Leng (1974) and the increased plasma concentrations of urea described by Parkins et al (1973), Roseby and Leng (1974) and Dargie (1980). These in turn explain the elevated rate of urea excretion (Roseby, 1973; Roseby and Leng, 1974) which accounts for a major component of the increased urinary N loss recorded in ostertagiasis (Parkins et al, 1973; Sykes and Coop, 1977), trichostrongylosis (Roseby, 1973; Roseby and Leng, 1974; Sykes and Coop, 1976; Steel et al, 1980; Poppi et al, 1981) and haemonchosis (Dargie, 1980; Abbott, 1982).

In summary, it can be stated that in trichostrongylidosis there is considerable loss of endogenous protein into the GI tract at the site of infection, and the nitrogen therein is largely reabsorbed further along the tract. Some of the nitrogen is absorbed as ammonia, converted to urea, and excreted in the urine, and thus does not represent a useful N source to the host. This loss of N contributes to the reduced N retention commonly observed in parasitised animals relative to uninfected controls. However, as the following discussion shows, alterations in the turnover of blood and tissue proteins also occur and these may adversely affect the efficiency with which absorbed amino acid nitrogen is utilised.

Studies have shown that the host responds to loss of blood

proteins into the GI tract by increasing the rate of synthesis and turnover of these proteins (Holmes and Mclean, 1971; Dargie, 1973; 1980; Steel et al, 1980; Abbott, 1982). The response, of course, may not compensate completely for the losses in which case the circulating levels and/or pool sizes of the various blood proteins will decrease.

In particular, hypoalbuminaemia often develops (Barker, 1973b; Reveron et al, 1974b; Sykes and Coop, 1976 & 1977; Steel et al, 1980; Abbott, 1982). Dargie (1975) suggested that parasitised sheep have a limited capacity for increasing albumin synthesis, particularly when N intake is depressed.

There is often an increase in circulating levels of globulins (Steel et al, 1980; Abbott, 1982), particularly IgG (Cripps and Steel, 1978). If, as Steel et al (1980) assume, there is no differentiation between albumin and globulin molecules at the site of loss, then it must be concluded that globulin synthesis continues at a greatly elevated rate throughout these infections.

The development of anaemia in haemonchosis has already been described and, as with plasma proteins, red cell loss is accompanied by increased synthetic rates. In animals of very low nutritional status, haematopoeitic reserves may become exhausted, and a deficiency-type anaemia will then supervene (Dargie and Allonby, 1975; Abbott, 1982).

It seems apparent that in the face of increased requirements for blood proteins, the infected animal will be required to divert its finite resources of amino acids and energy to this function at the expense of other less essential proteins. Symons and co-workers investigated this possibility in a series of experiments in animals

infected with T. colubriformis.

In studies using guinea pigs infected with T. colubriformis Symons and Jones (1981; 1983) examined the effect of infection on the partitioning of amino acid utilisation for protein synthesis in different organs and tissues. They found that the incorporation of ^{14}C-L-leucine was greater in the GI tract and liver of infected animals, and this was apparently at the expense of protein synthesis in skeletal muscle.

In earlier experiments (Symons and Jones, 1975; 1978) they showed that inappetance was responsible for reduced muscle protein synthesis, since it occurred equally among infected and pair-fed sheep. However, in a later experiment (Jones and Symons, 1982), the fractional synthetic rate and protein synthesised per day in skeletal muscle of lambs were both reduced by infection but were not reduced in pair-fed controls. In the same experiment they calculated whole body protein synthesis by measuring ^{14}C-L-tyrosine flux. When allowance was made for the protein ingested, or when expressed on a bodyweight basis, tyrosine flux in infected lambs exceeded that of the controls.

Steel and Symons (1982) discussed two possible explanations for the apparent increase in protein synthesis by infected sheep. Firstly, they considered that increased protein synthesis by the GI tract may be responsible. Secondly, they suggested that the higher tyrosine flux in such sheep may, in part, result from a higher rate of amino acid catabolism rather than increased protein synthesis, citing as evidence the concomitant increases in tyrosine flux and plasma urea concentration observed by Jones and Symons (1982). While it seems likely that both suggestions are in fact true, the

relative contributions of the two factors remains unresolved.

In conclusion, it can be stated that due to inappetance, enteric losses of protein and their replacement, there is a diversion of the limited supplies of amino acids available to infected animals from muscle and wool protein synthesis to processes essential for survival.

1.4.2 Energy metabolism

It is clear that energy metabolism is adversely affected in trichostrongylidosis, but the reasons for this remain unresolved. Reduction in feed intake is a major factor limiting the availability of energy for maintenance and, or, growth, but studies with pair-fed animals demonstrate that this is not the only reason for reduced energy retention in these animals.

Small reductions in apparent energy digestibility have been reported (Sykes and Coop, 1977; MacRae et al, 1982) but the major effect of trichostrongylosis is to lower the efficiency of use of digested energy (Sykes and Coop, 1976 & 1977; Dargie, 1980; Abbott, 1982). In some experiments increased urinary energy (as a result of increased urinary urea - see above) was a feature and this reduced the ME available to the animal. However, it is apparent that in many cases there is a reduction in the efficiency of utilisation of ME. For example, Sykes and Coop (1976; 1977), in comparative slaughter trials previously mentioned, found that the gross efficiency of use of metabolisable energy (ME) for growth was reduced by 37% in animals infected with T. colubriformis and 30% in those infected with O. circumcincta compared to pair-fed controls.

A likely explanation for the reduction in efficiency of utilisation of ME is increased heat production which one might

hypothesise would accompany the increased protein turnover that was discussed in the previous section. There are, however, no accurate figures for the energy cost of protein synthesis. Sykes (1983) assumed a value of 30 kJME/g protein synthesised, and calculated that the reduction in energy retention reported by Sykes and Coop (1976) in lambs infected with T. colubriformis would be accounted for by the additional synthesis of about 30 g protein per day. This, he suggested, was in reasonable agreement with the findings of Poppi et al (1981) that endogenous N loss was increased by 5 g per day, and the estimate of Jones and Symons (1982) that an extra 50 g protein per day was synthesised in the GI tract tissues.

It should be possible to measure this energy cost as increased heat production, but the few calorimetry studies conducted on parasitised animals have not tended to show such an increase. MacRae et al (1982), using a closed-circuit calorimeter, found that infection with T. colubriformis had no effect on heat production. In their experiment, altered energy retention was caused by increased faecal energy losses. Similarly, in calves given a mixed trichostrongylid infection (Randall and Gibbs, 1981), reduced energy intake and digestibility and increased urinary energy led to ME intakes being significantly lowered. Although the energy balance was reduced, and in clinically affected animals was negative, no differences in heat production were observed as a result of infection.

Steel (1972) measured the rate of irreversible loss of CO_2 from blood in sheep infected with T. colubriformis, and pair-fed and ad libitum controls, and concluded that, apart from a reduction associated with decreased feed intake, the energy expenditure of

sheep was not markedly affected by the parasites.

1.4.3 Mineral metabolism

There has been considerable interest in Ca and P metabolism in trichostrongylidosis over the past ten years. Reveron et al (1974a) and Sykes et al (1975) demonstrated that lambs infected with T. colubriformis showed reduced P and Ca retention, hypophosphataemia but normocalcaemia, and a virtual cessation of bone growth with some demineralisation. Histological examination of bones from infected sheep (Sykes et al, 1975) revealed a reduction in osteogenesis resulting in a generalised osteoporosis. The authors attributed the changes in infected sheep to impaired mineral absorption, particularly absorption of P, and proposed a secondary role for protein deficiency or undernutrition.

In lambs infected with O. circumcincta (Sykes and Coop, 1977; Sykes, Coop and Angus, 1977; Coop, Sykes and Angus, 1977; Coop et al, 1981), the principal findings with respect to Ca and P metabolism were slight reductions in Ca and P retentions, slight hypocalcaemia with normophosphataemia, and reductions of Ca and P deposition in the skeleton and whole body to about half that of pair-fed or ad-lib controls. The authors suggested that the skeletal changes in ostertagiasis probably resulted from deficiencies of protein and energy induced by the parasites leading to a matrix osteoporosis (Sykes et al, 1977).

The suggested causes of the skeletal abnormalities in trichostrongylosis (Sykes et al, 1975) and ostertagiasis (Sykes et al, 1977) summarised above are largely borne out by more recent work by Wilson and Field (1983). These authors used radioactive Ca and P and digesta markers in lambs fitted with abomasal and ileal

cannulae to measure the absorption and secretion of Ca and P in trichostrongylosis and ostertagiasis. They found that the true absorption of P was reduced in trichostrongylosis, and there were also increased losses of endogenous P and Ca as a result of this infection. The induced P deficiency led to a reduction in the plasma P concentration and reduced salivary flow of P. The effect on Ca metabolism was limited to an increase in endogenous faecal excretion. In contrast, the only effect of O. circumcincta infection was a small increase in endogenous faecal Ca excretion, with P absorption and secretion being unaffected.

1.4.4 Endocrine responses to trichostrongylidosis

In view of the diversity and extent of effects of trichostrongylid infections on protein, energy and mineral metabolism, it seems possible that the effects of the parasites, or the host's response, may be mediated by endocrinological changes. With the exception of the gut hormones, which have been discussed above, little attention has been paid to this field of study.

Prichard et al (1974) measured plasma concentrations of corticosteroids, insulin and total thyroxine (TT4) in sheep infected with T. colubriformis. They found that corticosteroid levels rose in both infected and pair-fed sheep, although the change was greater in infected animals. Insulin was depressed in both groups when feed intake fell. TT4 levels fell in infected sheep, but not in controls. The authors considered that the changes in insulin and corticosteroid concentrations were consistent with the changes in muscle and liver protein metabolism which occur in trichostrongylosis, i.e. increased liver and decreased muscle protein synthesis. The fall in TT4 concentration aroused some discussion.

Over 99% of plasma TT4 is protein-bound, and as hypoproteinaemia is a common feature of trichostrongylosis, the reduction in TT4 observed in the affected animals may have merely reflected reduced circulating total protein. Since it is the unbound fraction (free thyroxine, FT4) which is considered to be metabolically active, and this was not measured, no conclusions could be drawn from this experiment as to the role of thyroxine in trichostrongylosis.

The authors subsequently measured TT4 and FT4 in sheep infected with T. colubriformis (Hennessy, Prichard and Griffiths, 1977). While the TT4 values were again reduced, the infection had no effect on the concentration of circulating FT4.

In a further experiment (Hennessy and Prichard, 1981) they demonstrated that sheep infected with T. colubriformis, or pair-fed, developed an iodine (I) deficiency secondary to the anorexia caused by the parasite infection. In addition, the infected sheep but not the pair-fed controls were losing large quantities of protein-bound thyroxine into the GI tract, for which the thyroid gland, depleted of I, was unable to compensate. Nonetheless, unspecified homeostatic mechanisms maintained normal concentrations of plasma FT4 and, presumably, normal thyroxine function in these sheep.

1.5 Introduction to experimental work

The experiments presented in this thesis were conducted to examine further the effects of trichostrongylid parasites on production parameters and aspects of host metabolism.

The work described in Sections 3 and 4 examines interactions between nutrition and infection with *H. contortus*, furthering the work of Abbott (1982). In addition to observations on pathophysiological changes in animals subjected to different infection patterns, a considerable study is presented of the consequences of infection on feed conversion and body composition. Data on endocrinological changes is also included.

A study of the partitioning of dietary energy in sheep infected with *H. contortus* using indirect calorimetry is described in Section 5. This aspect of the relationship between the host and *H. contortus* has not been previously reported.

Three experiments described in Section 6 comprise an investigation into possible reasons for the relatively poor parasite establishment observed previously, particularly in the calorimeteric study. Separate experiments assess the pathogenicity of the laboratory strain of *H. contortus* and the possible anthelmintic effect of ammonium molybdate and sodium sulphate which had been administered to help prevent copper toxicity. A third study examines the susceptibility of comparable sheep to infection with *H. contortus*, and their ability to respond to vaccination with radioattenuated larvae.

Whereas the previous experiments were concerned with the effects of trichostrongylid parasites on parasite-naive growing lambs, Section 7 presents a study of the pathophysiological consequences of

prolonged challenge of mature immune ewes with O. circumcincta larvae.

SECTION 2

General materials and methods.

Section 2

General materials and methods.

2.1 Experimental animals

The sheep used in the various experiments were Suffolk x Greyface (sire Suffolk, dam Border Leicester x Scottish Blackface) and Scottish Blackface.

2.1.1 Management before the experiments

With the exception of the sheep in Experiments 6a and 7, the sheep used in the experiments were bred and reared at Cochno Farm, University of Glasgow.

The lambs were born indoors to ewes previously treated with the anthelmintic fenbendazole (Panacur Sheep Wormer: Hoechst) at housing in mid-pregnancy and at four weeks prior to lambing. The ewes and lambs were housed indoors on straw bedding. The lambs were tail docked and the males castrated a few days after birth. The lambs had continual access to the ewes' hay and from four weeks of age were offered a creep feed (140 g CP/kg FM) (Spillers Lambwena: Dalgety Agriculture Ltd., Glasgow, Scotland). This was changed, as required, to the appropriate experimental diet as described in each chapter. The lambs were weaned at eight weeks of age and kept in groups of suitable size until the experimental period.

2.1.2 Management during digestibility and nitrogen balance studies

Male lambs were moved to standard metabolism stalls (Duthie, 1959) to permit the separate collection of urine and faeces in order to measure feed digestibility and nitrogen (N) retention. Faeces

was collected in rubber faecal bags lined with 100 gauge polythene bags, or in 200 gauge polythene bags (Fishwick, 1973), held in place by a leather body harness. Urine passed through the metal-mesh floor of the cage and was collected via a chute in plastic vessels which had been acidified previously with 100 ml 5N HCl in order to prevent volatile N losses.

Daily routine

Nutritional balance studies were conducted over seven-day collection periods which were immediately preceeded by five-day adjustment periods wherein the sheep became familiar with the stalls and feed intakes were stabilised. The exception to this procedure is described in Section 5, where each collection was made over four days.

The lambs were offered their daily ration in one feed at 1000 h. Each morning, prior to feeding, any refusal or spillage of food from the previous day was weighed and recorded. Samples of feed and residues were retained for analysis. Wherever possible, feed levels were selected which minimised the likelihood of refusals. Each pair-fed control lamb was offered the amount of feed consumed by its infected partner at the same stage in the adjustment and collection periods.

The composition and quantities of the diets used in each experiment are described in the relevant Sections.

Five litres of fresh water (or more if required) was offered daily to each sheep and, in most experiments, their individual consumption was recorded.

Faecal bags were removed each morning before feeding and the total daily faecal output was recorded. The faeces was retained and

stored in tightly closed polythene bags and the seven day total collection was thoroughly mixed and subsampled at the end of the week.

Total daily urinary output was measured volumetrically or by weight, and either 10% or 100% was stored in screw-topped plastic containers until the end of the collection period when subsampling for laboratory analyses was performed.

Preparation of feed, faecal and urine samples for analysis

Samples of the pelleted diets were taken for analysis at appropriate intervals throughout each experiment. Feed refusal samples collected during nutritional balance studies were also retained for analysis.

Faeces collected from each sheep during the collection periods was well mixed and a subsample of approximately 500 g was retained for drying and subsequent proximate analyses. A further subsample of about 100 g was mixed to a slurry with water and analysed for N content.

Bulked urine from each sheep was well mixed and duplicate 100 ml aliquots were taken and stored at -20^{o}C for later determination of N content.

2.2 Chemical analyses of feed, faeces and urine

All the analytical methods used were officially established procedures (see MAFF, DAFS & DANI, 1981).

Dry matter (DM) The dry matter in food and faecal samples was determined by heating known quantities (400 - 500 g) in a forced hot-air oven at 80^{o}C for 48 to 72 h until constant weight was attained.

Gross energy (GE) The energy content of dried feed and faeces

samples was measured in an automatic adiabatic bomb calorimeter (Gallenkamp Autobomb).

Total nitrogen (N) Total nitrogen in dried food and wet faeces and urine samples was measured by a semi-automated Kjeldahl technique (Kjell-Foss Automatic 16210). Crude protein was calculated as

N x 6.25.

Crude fibre (CF) The crude fibre content of dried food and faeces samples was determined using the semi-automated Fibertec system (Tecator Fibertec 1010 Heat Extractor).

Ether extract (EE) The oil content was measured by extraction with petroleum spirit under controlled conditions.

Ash Ash content was the residue remaining after the sample was heated at 500^{o}C in a muffle furnace overnight.

Phosphorus (P) and Calcium (Ca) Phosphorus was measured spectrophotometrically, and calcium using an atomic absorption spectrometric technique.

Organic matter (OM) Organic matter was calculated as the ash-free dry matter.

Nitrogen free extract (NFE) The nitrogen free extract was calculated as follows:

NFE = DM - (Ash + CP + CF + EE)

2.3 Nutritional calculations

2.3.1 Calculation of digestibility coefficients

"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).

Digestibility is commonly expressed in terms of dry matter as a

coefficient:

$$\text{DM digestibility coefficient} = \frac{\text{DM in food} - \text{DM in faeces}}{\text{DM in food}}$$

The coefficients for each component of the DM, e.g. CP, OM, CF, Ash, and for energy can be calculated in the same way.

As discussed in Section 1, it is an over-simplification to state that the proportion of food not excreted in the faeces is equal to that which is absorbed from the digestive tract. In the case of nitrogen digestibility, the presence of metabolic faecal nitrogen in faeces, from sources such as sloughed epithelial cells, mucus and enzymes, results in an under-estimation of the proportion of dietary 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. In ruminants, methane arising from the fermentation of carbohydrate in the fore-stomachs is lost by eructation and thus is not absorbed. This results in an over-estimation of the digestible carbohydrate and digestible energy of the food. Consequently, the values obtained in digestibility studies are correctly termed "apparent" digestibility coefficients.

2.3.2 Nitrogen balance

In this thesis, N balance is expressed as g N retained or lost per day, and was calculated as follows:

N balance (g) = N in feed - N in faeces - N in urine

2.3.3 Water balance

The apparent retention of water per day was calculated as:

Water balance = water intake - water in urine - water in faeces

This equation takes no account of evaporative water loss, and

therefore the calculated water balance is more correctly termed an apparent retention (or loss) of water.

2.4 Carcase evaluation

2.4.1 Slaughter and preparation of samples

Where carcase evaluation studies were conducted, lambs were killed by captive bolt or electro-stunning followed by immediate exsanguination via the large vessels in the neck. The blood was collected in a separate bin for each sheep. The carcases were then dressed in the normal way, with the total "non-carcase" components of the body being retained for subsequent analyses.

The gastrointestinal tract was emptied of contents and the abomasal mucosa scraped as described in Section 2.6.5. The emptied gut, other viscera, halved head, lower legs and sheared pelt were added to the bin containing the blood from the relevant sheep and weighed prior to mincing. Unfortunately, it did not prove technically possible to include wool in the analysis.

The wet dressed carcase was weighed, allowed to set for 48 hrs at $+4^{o}C$, and then halved longitudinally through the vertebrae and sternum. The right half of each carcase was used for analysis. The "best end neck" joint was removed from each right half carcase for dissection after which the dissected pieces were returned to their respective half carcase. The remainder of the carcase was sawed into pieces not exceeding 20 x 10 x 10 cm. The weight of the pieces from each sheep was recorded prior to mincing.

2.4.2 Preparation of minced samples

Non-carcase components were minced 24 hrs after slaughter, and carcase pieces 48 hrs later.

An industrial machine was used to reduce the samples to a

uniform mince (Model F46: Karl Schnell GmbH & Co., Maschinenfabrik, 7065 Winterbach, Austria). The samples were minced twice, first through a plate with 15 mm perforations and then through a 5 mm die. The machine was cleaned between samples and when the die plate was changed. Carry-over between samples was kept to a minimum, and in order to minimise the effect on subsequent analyses, the samples from each experimental group were minced consecutively.

The samples were mixed well after each mincing and subsamples were collected after the second mixing. Duplicate aliquots of 150 g were retained for freeze-drying and subsequent chemical analyses. In the experiment described in Section 3 approximately 2 kg was pressed into a square polypropylene mould for neutron activation analysis. All samples were stored at -20^{o}C until analysis.

2.4.3 Dissection of the indicator joint

The "best end neck" (i.e. 7th to 10th rib) joint was selected for dissection. This was a modification of the method of Kempster, Avis, Cuthbertson and Harrington (1976) who showed that the composition of the "shoulder" (i.e. 7th to 12th rib) joint was a reliable index of carcase composition.

The joint was removed from the right half of each carcase after chilling for 48 hrs. The ribs were trimmed to one-third of their full length, parallel to the longitudinal cut through the vertebrae. The joint was weighed and then dissected into fat, muscle and bone, to the nearest gram. The weights of these components, and of the "eye muscle" (m. longissimus dorsi) were recorded. A sample of approximately 5 g of muscle was removed from deep within the eye muscle and this was freeze-dried to determine its dry matter content. The remainder of the joint was returned to the half carcase prior to

mincing.

2.4.4 Chemical analyses of minced samples

Samples of frozen carcase and non-carcase mince from each sheep were dried to constant weight in a freeze dryer (Edwards EF4 Modulyo freeze dryer: Edwards High Vacuum, Crawley, England). The dried samples were finely chopped in a liquidiser (ATO-MIX, M.S.E.) and subsamples of this were analysed for N, energy and ether extractable material. The fat-free residue from the ether extraction process was analysed for ash, Ca and P. The methods used were those outlined in Section 2.2 above. The results were expressed as g (or MJ) per kg of the original sample.

2.4.5 Calculation of body composition and gain

The quantities of each fraction in the original carcase (c), non-carcase (nc) components and, added together, the whole body, were calculated.

For example, total body gross energy (TBGE) was calculated as follows:

$$\text{TBGE (MJ)} = GE_c \text{ (MJ/kg DM)} \times DM_c \times \text{carcase wt (kg)} + GE_{nc} \text{ (MJ/kg DM)} \times DM_{nc} \times \text{non-carcase wt (kg)}$$

The total body protein (TBP), fat (TBEE) and water (TBW) values were calculated similarly.

Linear regression analysis of TBGE, TBP, TBEE, TBW and the empty body weight (Y), against bodyweight (BWt) at slaughter (X) of the initial control sheep in each experiment produced equations of the form:

$$Y = b \times X + a$$

Into these equations were substituted the initial BWt of each of the experimental sheep to derive initial body content data, which, when subtracted from the final body composition measured at slaughter, allowed calculation of the gain in each constituent over the experimental period.

2.4.6 Calculation of feed conversion and retention of dietary energy and crude protein

Feed conversion (%) was calculated as the gain in BWt over the experimental period, divided by the dry matter consumed, x 100.

Similarly, the percentage retention of dietary GE was calculated as the gain in energy content of the empty body, divided by the GE consumed over the course of the experiment, x 100, and the percentage retention of dietary crude protein was the gain in body protein divided by the CP consumed, x 100.

2.5 Radioisotopic studies

In different experiments various combinations of a number of radioisotopic preparations were used. These were ^{125}I-labelled albumin, ^{51}Cr-labelled red blood cells, $^{51}CrCl_3$, and ^{59}Fe-labelled transferrin. The particular preparations used in each experiment are described in the relevant section. In most respects the experimental design was similar regardless of which isotopes were being used, with some variation in the number of samples and the time at which they were taken.

2.5.1 Daily routine

The sheep were maintained in metabolism stalls during radioisotopic study periods. When radioactive iodine was to be injected, 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 radioisotopes and continued throughout the study. Blood samples and sub-samples of the total daily faecal and urinary outputs of each animal were collected regularly for the measurement of radioactivity.

2.5.2 Labelling techniques

^{51}Cr-labelled erythrocytes

Approximately 20 ml whole blood from each lamb was 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 radioiron. The cells were re-suspended in isotonic saline by gentle mixing. An appropriate amount of $Na_2{}^{51}CrO_4$ in isotonic saline (specific activity 37 MBq/ml, chromium content 5.4 ug/ml: Amersham International plc, Amersham, England) was divided amongst the blood

samples, each sample receiving approximately 37 MBq of ^{51}Cr.

The isotope and cell suspensions were mixed gently, then incubated at 37°C for 30 minutes to allow labelling to occur. After this the samples were centrifuged at 550 g for 10 minutes and the supernatant removed and discarded. The cells were then washed twice in isotonic saline to remove the unbound ^{51}Cr and were finally resuspended in isotonic saline ready for injection. The injection volume was approximately 15 ml. Each sheep received its own labelled red cells.

^{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 570 MBq/mg iron: Amersham International plc) in order to label plasma transferrin. Each sheep received approximately 6 MBq ^{59}Fe.

^{51}Chromic chloride

An approprate amount of $^{51}CrCl_3$ (specific activity 10 GBq/mg: Amersham International plc) was diluted 2:1 with isotonic saline. Each sheep recieved approximately 14 MBq ^{51}Cr in 7ml saline.

^{125}I-labelled albumin

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

Albumin Commercial sheep albumin was used (Sigma Chemical Company Ltd., Poole, Dorset, England) and a 2% solution prepared by

dissolving 600 mg of the freeze-dried protein in 30 ml isotonic saline.

Stock iodine monochloride solution This was prepared by dissolving 5.0 g potassium iodide and 3.22 g potassium iodate in 37.5 ml distilled water. 37.5 ml concentrated HCl and 5 ml carbon tetrachloride were added and the mixture shaken for several minutes. 0.1 M KI was added dropwise until a faint pink colour appeared in the CCl_4. This stock solution was diluted 1:350 with isotonic saline to give a solution containing 0.42 mg iodine/ml as iodine monochloride which was then used for the labelling procedure.

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 (185 MBq) 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 (Bovine Albumin Fraction V, Sigma Chemical Company) added to bring the specific activity of the protein to below 185 kBq and thus reduce the risk of denaturation of the ovine albumin. The labelled protein was dialysed for 48 hours against two changes of 20 l isotonic saline.

2.5.3 Preparation of standards

A known weight (approximate volume 1 ml) of each of the isotopically labelled preparations was emptied into a separate 100 ml volumetric flask. The contents of the flask were made up to the mark with 0.02 N NaOH. 1 ml aliquots of this solution were dispensed into counting vials and made up to 10 ml with 0.02 N NaOH. ^{51}Cr-labelled erythrocyte standards were prepared for each individual sheep and the ^{125}Iodine, $^{51}CrCl_3$ and ^{59}Iron standards were made up singly. The standards served as corrections against changes in the sensitivity of the counting equipment and for calculation of the injected dose.

2.5.4 Injection of isotopes

Known weights of the appropriate isotopic preparations were injected into each sheep via an intravenous cannula (internal diameter 1.19 mm, needle 14 G: Portex, Hythe, England) inserted in the right jugular vein. The cannula was flushed with isotonic saline after the isotopes had been injected and before withdrawing the cannula.

2.5.5 Collection and preparation of blood, faecal and urine samples for counting

Blood samples (5 ml) were collected from the left jugular vein into heparinised evacuated tubes at appropriate times after injection of the isotopes. For all isotopes, samples were taken at 10, 30 and 60 minutes after injection. When ^{59}Fe was used, additional samples were taken at 90, 120 and 180 minutes. Thereafter samples were collected at 1000 h daily 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 (if ^{51}Cr-red blood cells or ^{59}Fe-transferrin were in use) and 1 ml of plasma (for all isotopic

preparations) were pipetted into counting vials and made up to 10 ml with 0.02 N NaOH.

Total daily faecal and urinary outputs were recorded for each sheep. The daily faecal collection was well mixed then two samples of approximately 10 g were collected, packed to a volume of 10 ml in tared counting vials, and the weight of the samples recorded. A 10 ml sample of the daily urine output from each sheep was also retained for counting.

2.5.6 Radioactivity measurements

Count rates of the appropriate isotopes in the blood, plasma, urine and faeces were determined in an automatic well-type scintillation spectrometer (Packard Model 3300 Solid Scintillation Spectrometer: Canberra Packard, Pangbourne, Berkshire, England). The calculation of crossover factors was based on the relative count rates of the standard solutions of each isotope at each photo peak.

2.5.7 Calculation and expression of results

^{51}Cr-labelled RBC

The radioactivity of each blood sample was corrected for background radioactivity and crossover and expressed as counts per minute (cpm) per ml red cells using the PCV of each sample.

$$\text{cpm/ml RBC} = \frac{\text{cpm/ml of blood}}{\text{PCV}}$$

Apparent half-life (T 1/2)

When autologous ^{51}Cr-labelled red cells are injected into ruminants, there is a rapid loss of activity over the first few days due to elution. Once this rapid phase is complete, a slower exponential phase occurs and the red cell T 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 at ml/kg BWt.

$$\underset{(ml/kgBWt)}{RCV} = \frac{\text{Total injected } {}^{51}Cr \text{ activity cpm}}{\text{"Corrected" radioactivity of 1 ml RBC at } T_o} \div BWt(kg)$$

Gastrointestinal blood loss

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

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

^{59}Fe-labelled transferrin

T 1/2 and plasma iron turnover

A decrease in the plasma activity curve with time usually follows a single exponential curve when plotted on a semi-logarithmic scale. The radioactivity at the moment of injection is inferred by extrapolation back to the ordinate and the time taken for the plasma radioactivity to decrease to half the initial value (T 1/2) is obtained from 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 equations (Finch, Deubelbeiss, Cook, Eschbach, Harker, Funk, Marsaglia, Hillman, Slighter, Adamson, Ganzoni and Giblett, 1970).

$$\text{PIT (umol/l plasma per day)} = \frac{\text{Serum iron (umol/l)} \times 0.693^{*} \times 1440^{+}}{\text{T 1/2(min)}} \quad (1)$$

*Natural log of 2 +Number of minutes/day

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

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

PIT was then expressed as umol per day per kg BWt 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 two days post-injection until the end of the isotope study using the following formula:

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

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

Measurements were made of intestinal loss and reabsorption of iron after the method of Roche, Perez-Gimenez 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, ^{51}Cr faecal activity gives a measure of the total blood loss into the gut. With a knowledge of blood haemoglobin concentration, the amount of haemoglobin-iron (Hb-Fe) lost into the gut can be calculated:

$$\text{Hb-Fe loss into gut/d (mmol)} = \frac{\text{Hb (g/dl)} \times \text{blood loss } (^{51}Cr)\text{(ml)} \times 5.98^*}{100 \times 100}$$

* 100g Hb contains 5.98 mmol Fe

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

$$\text{Fe absorbed from gut (mmol)} = \text{Fe loss into gut } (^{51}Cr) \text{ (mmol)} - \text{Fe loss into gut } (^{59}Fe) \text{ (mmol)}$$

$^{51}\underline{CrCl_3}$

$^{51}CrCl_3$ was used to measure the daily loss of plasma (in particular, plasma proteins) into the GI tract. Insignificant amounts of $^{51}CrCl_3$ are reabsorbed from the GI tract, so the total faecal $^{51}CrCl_3$ radioactivity can be considered to equate with the loss of plasma into the GI tract (van Tongeren and Majoor, 1966; van Tongeren and Reichert, 1966).

$$\text{Plasma loss into GI tract (ml/day)} = \frac{^{51}Cr \text{ cpm in 24 h faeces}}{^{51}Cr \text{ cpm/ml plasma at start of 24 h}}$$

^{125}Iodine-labelled albumin

T 1/2

This was calculated from the plasma disappearance curve as for the other radioisotopes.

Plasma volume (PV)

This was calculated using the dilution principle and is expressed as ml/kg BWt:-

$$\text{PV (ml/kg BWt)} = \frac{\text{Total injected } ^{125}\text{I activity (cpm)}}{\text{Corrected activity of 1ml plasma at } T_o} \div \text{BWt (kg)}$$

Intravascular pool of albumin

This was obtained by multiplying the plasma volume (l) by the albumin concentration (g/l) and dividing the result by the BWt (kg).

$$\text{CA (g/kg BWt)} = \frac{\text{PV (l) x serum albumin (g/l)}}{\text{BWt (kg)}}$$

Total body albumin (TA)

This was calculated using the "equilibration time" method described by Campbell, Cuthbertson, Matthews and McFarlane (1956) which depends on the daily radioactivity excreted in the faeces and 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, Q_E was maximal, and thus the ratio of Q_E to Q_P was assumed to be equal to the ratio of their pool masses, i.e.

$$\frac{Q_E}{Q_P} = \frac{EA}{CA}$$

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 as the difference between TA and CA.

Fractional catabolic rate (k)

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

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

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 exist in parasitised animals (Dargie, 1975).

2.6 Parasitological techniques

2.6.1 Culture and harvesting of H. contortus and O. circumcincta larvae

Pure cultures of H. contortus or O. circumcincta were prepared by the following method.

A culture lamb reared parasite-free was infected with a pure culture of larvae of the required species and once eggs were detected in the faeces, the lamb's daily faecal output was collected in rubber bags. The faeces was incubated in glass jars with loosely fitting lids at room temperature for 10 to 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 material. The fluid containing the larvae was filtered through a filter paper (Grade 113: Whatman Ltd, Maidstone, Kent, England) by suction using a Buchner apparatus and vacuum pump. The filter paper was then removed and inverted onto an eight inch milk filter (Maxa Filters: A. McCaskie, Stirling, Scotland) 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 luke-warm water. The motile larvae were left to migrate through the milk filter into the water below. They accumulated at the neck of the funnel and were collected after eighteen hours.

2.6.2 Preparation 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. A variation of ± 10% of the desired concentration of larvae was attained. If the larvae were not to be used immediately, they were stored at +4°C until required.

2.6.3 Administration of larval doses

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

2.6.4 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; MAFF, 1971).

2.6.5 Worm burdens at slaughter

The lambs were slaughtered either by intravenous injection of pentobarbitone sodium (200 mg/ml) (Euthatal: RMB Animal Health Ltd, Dagenham, Essex, England) or by captive bolt or electro-stunning and immediate exsanguination. The abomasum was then removed for assessment of the parasite burden. 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 examination. 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 digesta were then diluted to 2 litres, mixed well and 2 x 200 ml samples were formalised and retained for worm counting.

To each 200 ml aliquot of abomasal washings and digesta, 2 - 3 ml iodine solution (720 g potassium iodine and 450 g iodine crystals/l distilled water) was added and mixed thoroughly. Ten 4 ml aliquots were withdrawn by 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.

In the case of H. contortus, it was found that this counting technique did not give reproducible results because the adult worms are large and heavy and it was difficult to ensure satisfactory mixing within the jar to obtain representative 4 ml samples. Therefore when this worm was present the entire 200 ml subsample was examined, and the number of worms found multiplied by 10 to give the total worm burden of the sheep. It was found by examining duplicate 200 ml samples and the residual 80% of contents from several sheep that this method gave results reproducible to within ± 5%.

2.7 Haematological techniques

2.7.1 Collection of samples

Blood samples were collected by jugular venepuncture into evacuated 5 ml glass tubes (Vacutainer: Becton Dickinson Vacutainer Systems Europe, Meylan Cedex, France) containing anticoagulant. Lithium heparin (143 U.S.P units/tube) was used unless white cell counts were to be made, in which case di-potassium sequestrene (K_2EDTA) (10 mg/5 ml blood) was used since this latter anticoagulent does not cause the white cells to clump. Samples were gently inverted several times immediately after collection to ensure complete mixing.

2.7.2 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., England). 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 sheep red cells. In addition to providing the red and white cell counts, the values for haematocrit (or packed cell volume PCV) 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 microhaematrocrit method.

Calculation of the red cell indices

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

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

Mean corpuscular haemoglobin (MCH)

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

Mean corpuscular haemoglobin concentration (MCHC)

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

2.8 Biochemical techniques

2.8.1 Collection of samples

Blood for most biochemical analyses was collected into 10 ml iron-free Vacutainer tubes containing no anticoagulant. The samples were left at room temperature for 24 h before the serum was harvested. Serum was stored at -20°C for later analyses. Heparinised blood samples were used for haemoglobin typing. When plasma glucose concentration was required, samples were collected in tubes containing potassium oxalate and sodium fluoride to prevent further glycolytic activity.

2.8.2 Analytical techniques

Total serum protein, albumin, globulin and urea

Total serum protein, albumin and urea were measured by continuous flow analysis (Standard Technicon Auto-Analyzer II method). Globulin was calculated as the difference between total protein and albumin concentration.

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

Serum iron was determined spectrophotometrically using a commercial test kit (Roche Diagnostica, Welwyn Garden City, Herts., England). 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 percentage saturation of transferrin with iron was calculated as follows:-

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

Haemoglobin typing

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

Plasma glucose

The concentration of glucose in plasma was measured in a Beckman Glucose Analyser.

Plasma pepsinogen

A method similar to that described by Edwards, Jepson and Wood (1960) was used to measure plasma pepsinogen activity. Plasma was incubated at 37°C with bovine serum albumin substrate (Fraction V: Sigma Chemical Company Ltd) adjusted to pH 2. After 24 h, protein was precipitated with 4 % trichloroacetic acid (TCA) and the liberated phenolic amino acids (tyrosine) non-precipitable with TCA were estimated with Folin-Ciocalteau reagent (British Drug Houses, Poole, England). Readings were carried out using a spectrophotometer (Unicam, Cambridge, England). The enzyme activity was expressed as international units (iu) tyrosine (umol tyrosine

released per 1000 ml serum per minute).

Serum hormone concentrations

Serum concentrations of insulin, prolactin and growth hormone (somatotropin) were measured by double-antibody radioimmunoassays (Vernon, Clegg and Flint, 1981).

Cortisol concentration was determined using a commercial kit obtained from IRE-UK, High Wycombe, Bucks, England (see Flint, Clegg and Knight, 1984).

2.9 Statistical analysis

Results are presented as individual values, or as group mean (x) plus or minus the standard error of the mean ($\pm$ SE).

Statistical analyses were conducted using MINITAB and GLIM packages on an ICL 3980 mainframe computer, and using BASIC programs adapted from Barlow (1983) for use on a Commodore 64 microcomputer.

The statistical tests used in each experiment are described in the relevant sections.

Probabilities of $p < 0.05$ were considered significant.

SECTION 3

The effects of single or trickle infections with *Haemonchus contortus* on the growth, body composition and metabolism of lambs offered feed *ad libitum*.

Section 3

The effects of single or trickle infections with *H. contortus* on the growth, body composition and metabolism of lambs offered feed *ad libitum*.

3.1 Introduction

It is well-established that infection with *T. colubriformis* or *O. circumcincta* can affect the growth and body composition of lambs, and the evidence for this has been reviewed previously. Less attention has been paid to the effects of *H. contortus* infection, with the exception of an experiment by Abbott (1982). She conducted a limited study of carcase composition in sheep infected with *H. contortus* by dissecting an indicator joint, the 7th to 10th ribs or best end neck joint, and found a reduced percentage of muscle in infected sheep relative to controls, the difference being most marked among sheep fed a low protein diet.

The present experiments were designed to extend the work of Abbott (1982) and to measure the intake and utilisation of food by growing lambs infected with *H. contortus*, and the effects of the infections on certain metabolic parameters. The experiments share similarities in design with those of Sykes and Coop (1976 & 1977), which measured body compositional changes in lambs infected with *T. colubriformis* and *O. circumcincta* respectively.

Two patterns of infection were used. Lambs in the first experiment were given a single dose of 350 *H. contortus* larvae/kg, and those in the second were infected twice weekly with small numbers of larvae (20 larvae/kg). These two regimes simulate the field

situations from which acute and chronic haemonchosis develop respectively (Allonby and Urquhart, 1975).

Two groups of uninfected control lambs were used. Lambs of one group were pair-fed to individual infected lambs, while the infected and the remaining control lambs were offered feed ad libitum. By this means it was hoped to separate and assess the effects of parasitism on feed intake (ad libitum controls v infected) and on the utilisation of food (infected v pair-fed controls) (Sykes and Coop, 1976).

Previous workers have demonstrated that the haemoglobin type of the host can influence the pathogenesis of haemonchosis, with sheep of Hb type B being more susceptible than those of type A, and those of type AB being intermediate in susceptibility (Allonby and Urquhart, 1976; Altaif and Dargie, 1978; Preston and Allonby, 1979b). In allocating sheep to groups for the present experiments, account was taken of their haemoglobin types in order to achieve an equal distribution of types within the groups.

Clinical, parasitological, haematological and biochemical parameters were measured to assess the severity of disease resulting from the infections. The opportunity was also taken to measure the effect of infection on serum endocrine concentrations, an aspect of host metabolism not previously reported in animals infected with H. contortus.

The following two experiments respectively investigate the effects of a single moderate infection, and repeated small infections, with H. contortus on the growth, body composition and metabolism of lambs offered feed ad libitum.

Experiment 3a The effects of a single moderate infection with *H. contortus* on the growth, body composition and certain metabolic parameters of lambs offered feed *ad libitum*.

3a.2 Materials and methods

3a.2.1 Experimental design

Twenty, 10-week old lambs reared parasite-free were penned individually and their intakes of a complete pelleted ration monitored. The lambs were allocated to four groups of five lambs on the basis of Hb type and bodyweight. Five lambs were killed on day 0 of the experiment (group IC) and carcase analysis was carried out to provide values for energy, protein and fat content with which to compare lambs killed later. Of the remaining lambs, 5 were given a single infection of 350 *H. contortus* larvae/kg BWt, and offered feed to appetite (ALSI). A further five, PFSC, were paired to individuals of ALSI, and each offered the amount of feed eaten by its infected partner the previous week (pair-fed controls). The remaining five lambs, ALC, were additional uninfected controls, offered feed to appetite. The allocation of individuals to groups and the haemoglobin types of the lambs are shown in Table 3a.1.

Feed intake, haematological changes, faecal egg counts and bodyweights were measured weekly throughout the study. Aspects of blood biochemistry were measured; serum proteins, iron and iron binding capacity weekly, and growth hormone, insulin, cortisol and prolactin, and plasma glucose at prescribed intervals on the 31st day after infection. Nitrogen balance and digestibility studies were conducted on groups ALSI and PFSC six weeks after the infection date.

Table 3.a.1

The Haemoglobin (Hb) types of lambs infected with *H. contortus* (ALSI) and of uninfected controls killed at the start of the experiment (IC), pair-fed(PFSC) or offered feed *ad libitum* (ALC)

Group ALSI Sheep No.	Hb type	Group PFSC Sheep No.	Hb type
ALSI-17	B	PFSC-25	AB
ALSI-31	AB	PFSC-18	B
ALSI-7	B	PFSC-12	AB
ALSI-20	AB	PFSC-16	AB
ALSI-27	AB	PFSC-23	B

Group ALC Sheep No.	Hb type	Group IC Sheep No.	HB type
ALC-37	B	IC-45	AB
ALC-41	B	IC-6	AB
ALC-15	AB	IC-26	AB
ALC-3	AB	IC-5	B
ALC-19	B	IC-13	B

The sheep were killed 49 days after infection (DAI), and their abomasal worm burdens were assessed and carcase evaluation carried out. In this experiment, carcase evaluation included indicator joint dissection and chemical analysis of carcase and non-carcase homogenates, and the techniques used are described in Section 2. In addition, neutron activation analysis (NAA) was conducted on some samples of minced carcase and non-carcase in order to compare this technique with conventional chemical analysis.

Group PFSC lagged one week behind the other groups in terms of feed intake, time of nutritional studies and slaughter. This was to facilitate pair-feeding.

3a.2.2 Experimental animals

Twenty castrated male Suffolk x Greyface lambs were used. They had been born indoors to ewes previously treated with the anthelmintic fenbendazole (Panacur Sheep Wormer: Hoechst) at housing in mid-pregnancy and at four weeks prior to lambing. The ewes and lambs were maintained in large straw-bedded pens and the lambs had access to the ewes' hay. At four weeks of age the lambs were first offered a 14% protein commercial creep feed (Spillers Lambwena, Dalgety Agriculture Ltd). Two weeks later this was gradually changed over to the experimental diet. The lambs were weaned at eight weeks old. At an average age of 10 weeks (range 8 1/2 to 11 weeks) the lambs were moved to their experimental accomodation.

3a.2.3 Accomodation

The lambs were housed singly in individual calf-pens measuring 6' by 3' (Poldenvale Ltd, Williton, Somerset). The pens had solid wooden backs, metal barred sides, and fronts fitted with two rings to hold buckets for feed and drinking water. Peat moss was used as

bedding, and was thoroughly scraped out and changed twice weekly.

The lambs were moved to standard metabolism stalls for the digestibility and N balance studies.

3a.2.4 Feeding

The diet used in this experiment was a complete pelleted ruminant feed, Super Star Cubes (Hamlyn Milling, Balgarvie Mill, Scone, Perth). The feedstuff comprised approximately one quarter each of dried grass and barley, with smaller proportions of other grains, pulses, by-products and mineral and vitamin preparations. The proximate analysis of the diet is provided in Table 3a.2.

Sheep of groups ALSI and ALC were each offered feed to appetite twice daily at 0830 and 1630 h from their own bin of feed. The bins were filled and weighed at the start of each week and weighed again at the end of the week. The weight of feed consumed was calculated by subtraction, taking into account any spillage or wastage of feed. Each week the pair-fed animals were offered the weight of feed consumed by their infected partners the previous week, divided into seven equal rations offered once daily at 0830 h.

During the nutritional balance study, feed intake by the ad libitum sheep was measured daily, and their partners fed that amount one week later.

3a.2.5 Experimental infections

Sheep of group ALSI were each given a single oral dose of 350 H. contortus larvae/kg BWt on day 0 of the experiment. The doses were prepared as described in Section 2.

3a.2.6 Endocrine and glucose studies

Blood samples were collected from each sheep by jugular venepuncture into evacuated tubes at 0800, 0900, 1000, 1200, 1400,

Table 3a.2

Proximate analysis of Super Star cubes (Hamlyn Milling, Balgarvie Mill, Scone), a complete pelletted ruminant feed.

	Proximate Analysis
DM	889 g/kg FM
CP	136 g/kg DM
CF	140 g/kg DM
EE	35 g/kg DM
Ash	96 g/kg DM
GE	17.6 MJ/kg DM

1600, 1800 and 2000 h on day 31 after infection. Assays for serum cortisol, growth hormone, prolactin and insulin were conducted on each sample. Plasma glucose concentration was measured in samples taken at 0800, 0900, 1000 and 1200 h. The techniques involved are described in Section 2.

3a.2.7 Carcase evaluation

The sheep were killed, prepared and minced and suitable aliquots of mince were analysed for DM, GE, protein and EE. An indicator joint was dissected. Body composition and gains over the infection period were calculated, as were the percentage retentions of dietary gross energy and protein. The techniques and calculations are described in Section 2.

Linear regression analysis of TBGE, TBP, TBEE and the empty body weight (Y), against BWt at slaughter (X) of the five sheep of group IC produced equations of the form $Y = (B \pm \text{s.e. of } B) \times X + A$, shown in Table 3a.3.

3a.2.8 Neutron activation analysis

Neutron activation analysis (NAA) is a non-destructive technique for measuring quantities of various elements in a sample. Briefly, a substance to be analysed is bombarded with high-energy neutrons, a proportion of which collide with atomic nuclei in the sample to form isotopes. Many of these new isotopes are radioactive and decay in a characteristic fashion, yielding γ-rays of a particular half-life and energy. These γ-rays are measured, and compared to those given off by similarly irradiated standard solutions of elements. By proportion, it is then possible to quantify the amount of each element in the original sample.

The technique has long been used *in vitro* for the measurement

Table 3 a.3

Regression equations of the form $Y = (B \pm \text{s.e. of } B) \times X + A$

Where X = B Wt (kg)

and Y =

GE_{body} (MJ)	$y = (13.7 \pm 0.81)x - 118.7$	$t = 16.98$	$P < 0.001$
$Protein_{body}$ (g)	$y = (137.5 \pm 3.68)x - 71.3$	$t = 37.4$	$P < 0.001$
Fat_{body} (g)	$y = (263.2 \pm 27.2)x - 2919.1$	$t = 9.68$	$P < 0.01$
Empty B Wt (kg)	$y = (0.894 \pm 0.0449)x - 1.800$	$t = 19.91$	$P < 0.001$

of trace levels of elements in very small samples. In the last 20 years, a larger-scale adaptation has been developed to allow *in vivo* analysis of the human body and this has yielded useful data, not easily available otherwise, concerning body chemistry in health and disease. Anderson, Osborn, Tomlinson, Newton, Rundo, Salmon and Smith (1964) reported the first measurements of Na and Cl in man. Since then, other elements have been added to the repertoire and it is now possible to measure O, H, N, Ca, P, Na, Cl, Mg, I, Al, Cd and Ag, as well as K from the naturally-occurring isotope, ^{40}K (Cohn, 1980; East, 1982). With N, Ca, P, K, Na and Cl, precision better than $\pm$ 4 % is possible (Sharafi, Pearson, Oxby, Oldroyd, Krupowicz, Brooks and Ellis, 1983).

The major problem associated with applying the technique to different species is that the pattern of γ-ray emission detected by the monitor varies with the shape of the emitting body and the distribution of the elements within the body. To correct for this variation, 'phantoms' or body-shaped structures containing standard solutions of the elements to be measured are constructed and used to calibrate the equipment. In order to measure a different species, a new set of 'phantoms' is required, the preparation and testing of which is expensive and time-consuming.

Until recently, published reports of NAA of animals were restricted to a few papers concerning mice or rats. East, Preston and Robertson (1983) and Preston, East and Robertson (1984) described observations on body composition measurements of pigs and sheep as well as rats and humans.

The present comparison of NAA and conventional chemical analysis of minced sheep samples was intended as a fore-runner to a future

experiment where the body composition of infected and control sheep would be compared in vivo using NAA.

Blocks of mince, frozen in suitable polypropylene moulds, were prepared for NAA. The natural radioactivity of each block was measured for 1200 s in a fixed position in a shadow-shield human whole-body monitor. After this background count, the mince block was transferred to a 14 MeV neutron generator and irradiated for 300 s. The block was then returned to the monitor where two counts of 300 s and 900 s were made to measure N, Ca, P, Na & Cl from the induced radioactivity.

Initial calibration of the equipment was carried out with single and multielement solutions in polythene bottles and neutron fluence was normalised by irradiating four copper wires and counting the induced activity with each sample. The gamma spectra were corrected for Compton contributions, detector activation, annihilation radiation sources other than ^{13}N and interfering activities before computing the results.

3a.2.9 Statistical analysis

Parasitological data, being non-parametric, were analysed using the Mann-Whitney test. Other differences were analysed using the paired t-test or analysis of variance. The significance of regression equations derived from carcase composition data were tested using the t statistic, and those of other correlations using the correlation coefficient r.

3a.3 Results

3a.3.1 Clinical and bodyweight changes

All the lambs grew well for the first five weeks of the experiment, with no differences between the experimental groups

(Figure 3a.1). A decrease in bodyweight was observed in groups ALSI and PFSC, but not in ALC, during the nutritional balance period when the lambs were housed in metabolism stalls (35 - 49 DAI). This coincided with a drop in feed intake in group ALSI.

No adverse clinical signs were detected in any of the sheep.

3a.3.2 Parasitological findings

Faecal egg counts

Strongyle eggs were first detected in the faeces of the infected animals between 17 and 24 DAI (Figure 3a.2). Mean faecal egg counts peaked at 21,210 epg on day 43. However there was considerable individual variation in the size and time of peak egg excretion, with ranges of 9,000 - 58,000 epg and 24 - 43 DAI respectively. Total egg outputs were measured at day 43, and the individual results are presented in Table 3a.4.

Worm burdens

All worms recovered at slaughter were adult H. contortus. The mean burden was 2,648, which represented 32.6 % of the original larval challenge with a sex ratio (male/female) of 1.07. There was a significant positive correlation ($p<0.05$) between the total egg output on day 43 and the number of parasites recovered on day 49. Data for individual sheep are presented in Table 3a.4.

Uninfected sheep

No strongyle eggs were found in the faeces of the uninfected sheep, and no worms were found in their abomasa at slaughter.

3a.3.3 Nutritional studies

Feed intake

Feed intake increased in all the groups over the course of the experiment as the lambs grew (Figure 3a.1). Intake was generally

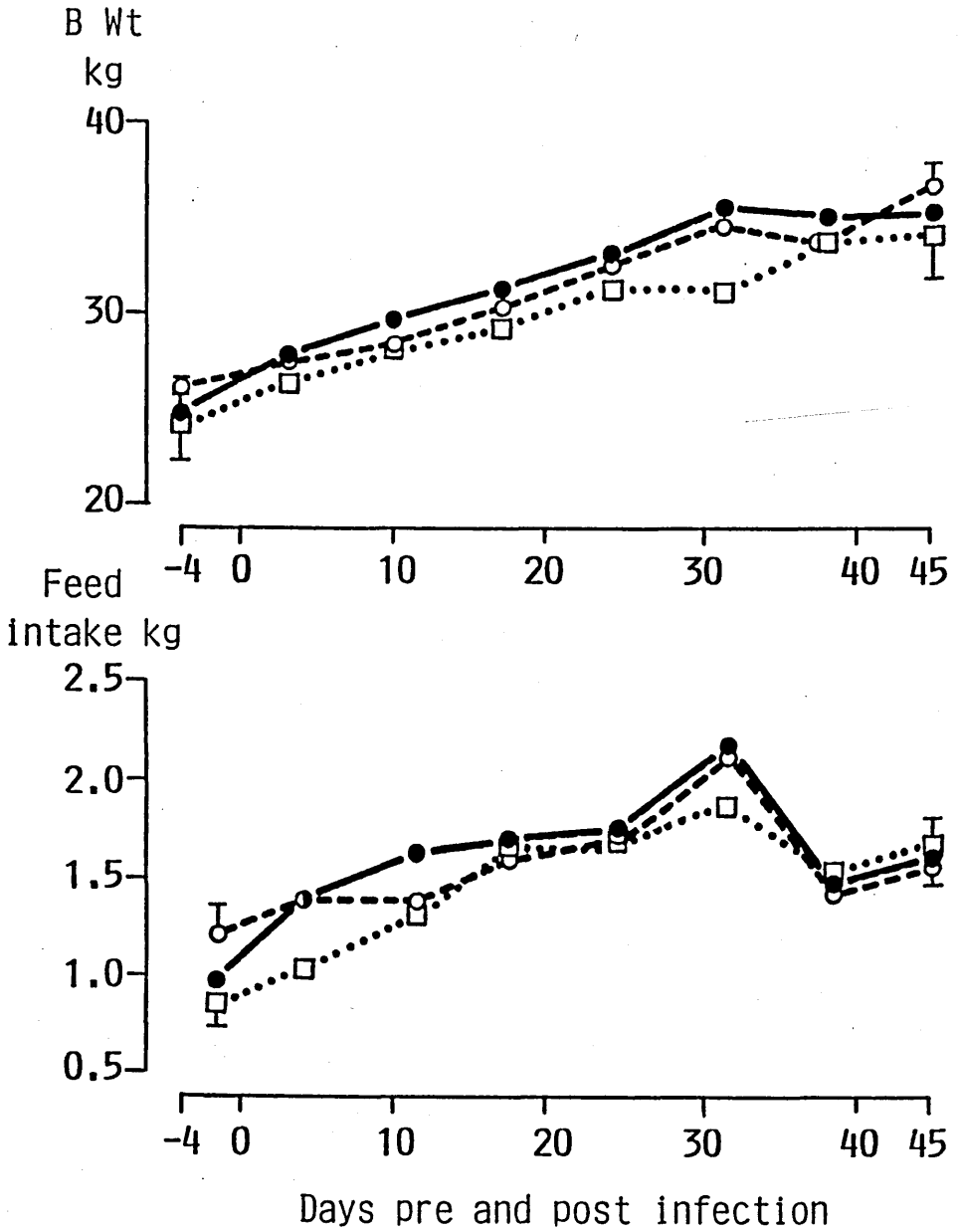

Figure 3a.1 Bodyweight (BWt) and daily feed intake of lambs infected with H. contortus (●———●) and controls pair-fed (O- - - -O) or offered feed ad libitum (□·····□).

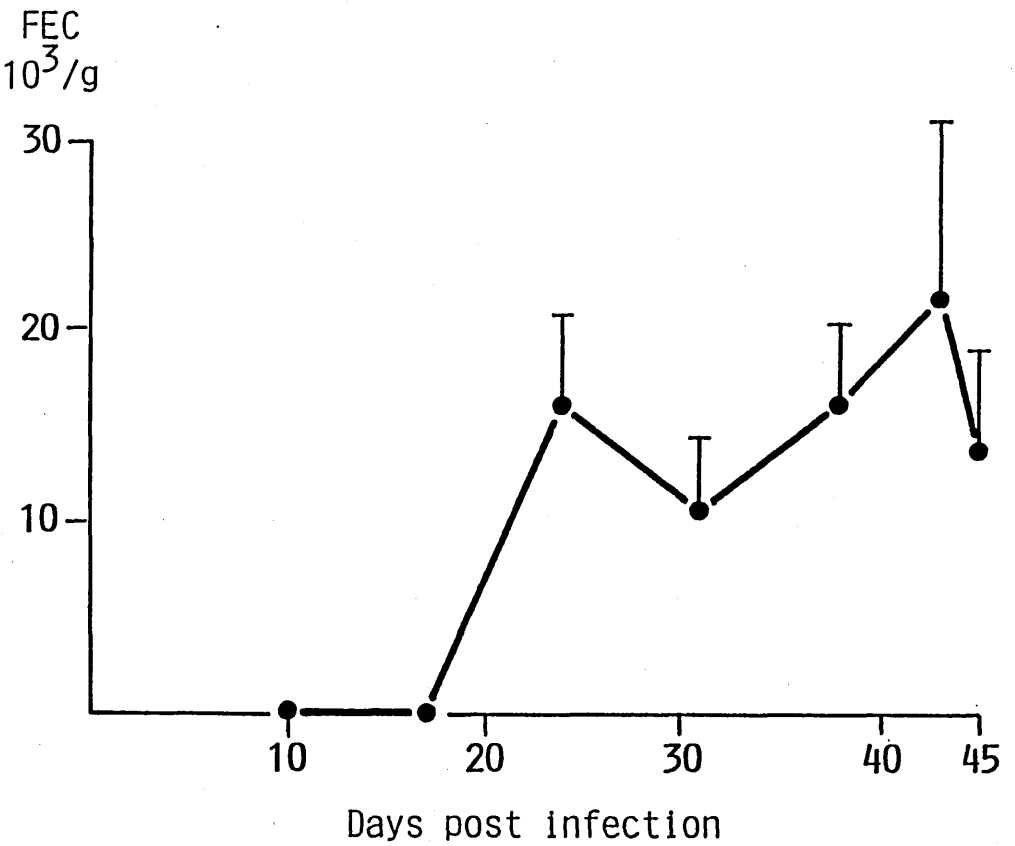

Figure 3a.2 Faecal egg count (FEC) of lambs infected with H. contortus (●——●).

Table 3.a.4

Individual worm burdens and total daily egg outputs of lambs infected with 350 *H. contortus*/kg BWt and killed 49 days after infection (DAI)

Sheep No.	Larval Challenge	Worms recovered 49 DAI			% Recovery	Total Egg Output 43 DAI
		Male	Female	Total		
ALSI-17	6,000	1,830	1,790	3,620	60.3	28,000,000
ALSI-31	7,000	650	570	1,220	17.4	10,800,000
ALSI-7	8,000	720	590	1,310	16.4	8,100,000
ALSI-20	9,000	970	1,340	2,310	25.7	29,000,000
ALSI-27	11,000	2,640	2,140	4,780	43.4	66,500,000
Mean (± S.E.)	8,200 (860)	1,362 (382)	1,286 (315)	2,648 (687)	32.6 (8.4)	28,500,000 (10,400,000)

highest in group ALSI, in which it peaked at 2.2 kg per sheep per day at 35 DAI. A sharp decline in appetite was observed when the sheep were placed in metabolism stalls, this decrease being most marked in group ALSI. Feed intake tended to recover after a few days in the stalls.

Digestibility trial

Group mean digestibility coefficients are presented in Table 3a.5. There were no differences in digestibility of DM, OM, EE, NFE or GE between the groups. The digestibility coefficients of crude protein and ash recorded in group ALSI (0.477 and 0.415 respectively) were significantly lower than those of group PFSC (0.533 and 0.444 for protein and ash respectively). In contrast the infected sheep had significantly higher coefficients of digestion of CF than the controls (0.234 and 0.197 respectively).

Nitrogen balance

All sheep were in positive nitrogen balance (Table 3a.6). The sheep of group ALSI had significantly lower N balances than those of group PFSC (3.9 and 6.6 g/day in the two groups respectively).

Water balance

There were no significant differences in water intake, urine volume, faecal water content or in water balance between the groups (Table 3a.6).

3a.3.4 Haematology and biochemistry

Since infection with H. contortus did not cause a reduction in voluntary feed intake, groups ALC and PFSC had very similar intakes and were effectively duplicate control groups. The results for most haematological and biochemical parameters have therefore been pooled, and the control figures presented represent 10 uninfected sheep.

Table 3a.5

Digestibility coefficients of lambs infected with 350 *H. contortus* larvae/kg BWt and their pair-fed controls

	DM	CP	CF	EE	Ash	NFE	OM	GE
Infected n = 5	0.617 (0.012)	0.477 (0.021)	0.234 (0.008)	0.761 (0.036)	0.415 (0.008)	0.763 (0.011)	0.640 (0.013)	0.595 (0.019)
Control n = 5	0.623 (0.007)	0.533 (0.011)	0.197 (0.017)	0.797 (0.014)	0.444 (0.013)	0.764 (0.007)	0.643 (0.007)	0.598 (0.006)
Significance		*	*		*			

*P < 0.05, paired t-test

Table 3.a.6

Mean (± S.E.) nitrogen and water balances of sheep infected with 350 *H. contortus*/kg BWt and their pair-fed controls.

	N Balance (g/day)		Water Balance (l/day)	
	Infected	Control	Infected	Control
n	5	5	5	5
Intake	29.5 (1.13)	30.7 (1.07)	3.9 (0.30)	3.8 (0.24)
Faeces	15.4 (0.85)	14.3 (0.40)	1.0 (0.11)	1.1 (0.11)
Urine	10.2 (0.94)	9.9 (0.95)	1.1 (0.18)	1.1 (0.16)
'Retained'	3.9 (1.19)*	6.6 (0.42)*	1.8 (0.13)	1.6 (0.09)

* $P < 0.05$, paired t-test

Haematological changes

The infected sheep developed a moderate macrocytic, normochromic anaemia. The mean PCV of group ALSI fell from 0.353 l/l prior to infection to 0.256 at 24 and 38 DAI and recovered slightly to 0.282 by 45 DAI (Figure 3a.3). Sheep ALSI-27 and ALSI-17 showed the greatest decreases in the group, their PCVs falling to 0.21 and 0.22 respectively. PCVs of the other three sheep in the group did not fall below 0.26 at any time. There was a slight drop in mean PCV among the control sheep over the course of the experiment, from 0.376 to 0.346. The mean PCV of group ALSI was significantly lower than that of the control sheep on every occasion from 17 DAI.

Haemoglobin and RCC values showed similar trends to the PCVs, with those of group ALSI being significantly lower than those of the control group from 17 and 24 DAI respectively . The mean Hb concentration of group ALSI fell from 12.7 g/dl prior to infection to 8.6 by 24 DAI, and then rose slightly to 9.5 at 45 DAI (Figure 3a.4). The mean RCC of group ALSI fell from 12.49 x 10^{12}/l to 7.30, then recovered slightly to 7.93 by the end of the experiment (Figure 3a.4). Values from the control sheep showed little change over the course of the experiment.

The mean MCV of the infected sheep rose from 28.4 to 33.6 fl over the course of the experiment and was significantly higher than the control group from 24 DAI ($p<0.05$) (Figure 3a.5). The mean MCH value of group ALSI dropped from 10.2 to 9.2 pg by 17 DAI and then increased to 12.2 pg by 45 DAI and was significantly higher than control values at 31 DAI ($p<0.05$) (Figure 3a.5).

The group mean MCHC value of group ALSI fell from 35.7 g/dl before infection to 32 g/dl by 24 DAI and then recovered to control

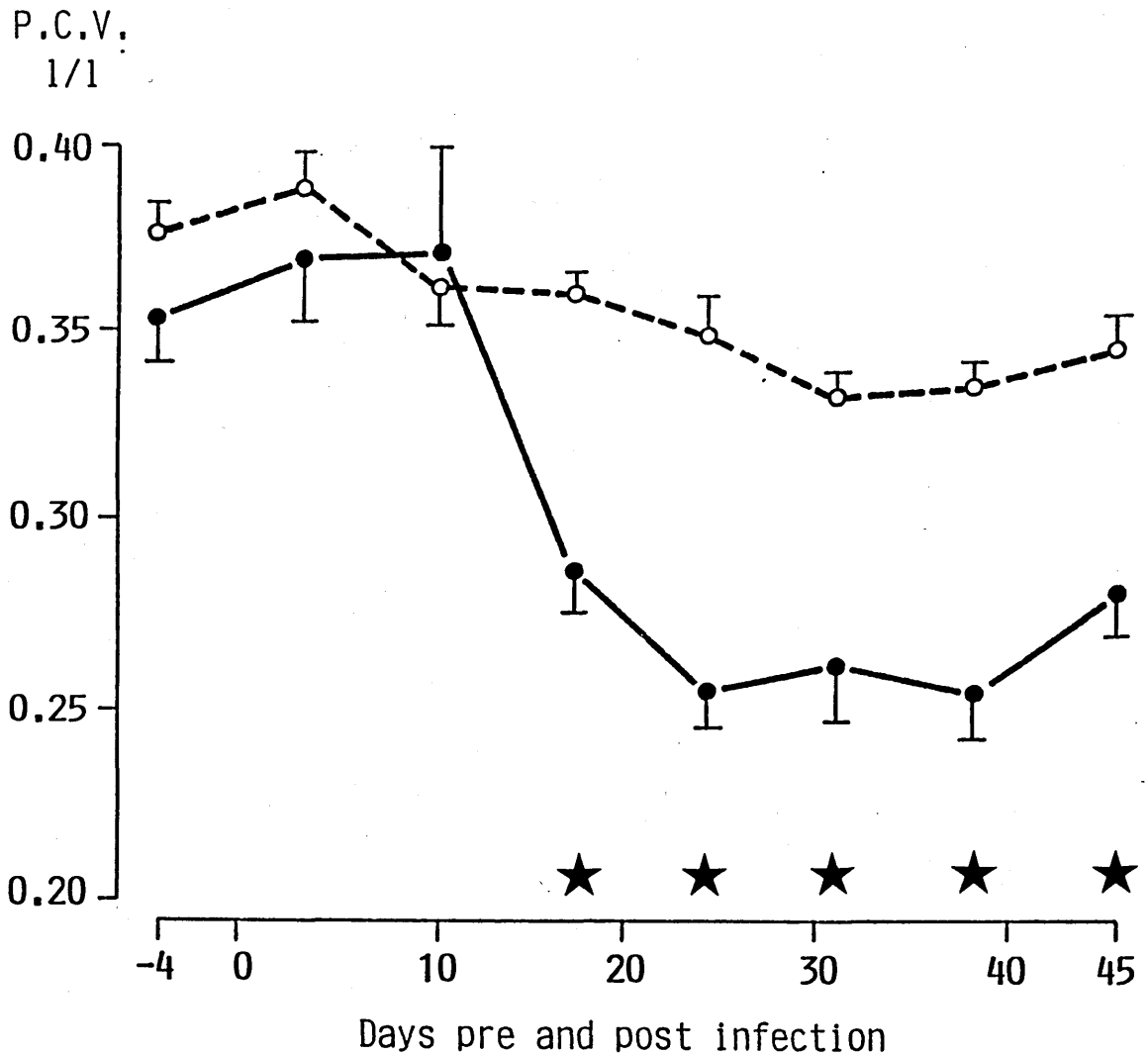

Figure 3a.3 Packed cell volume (PCV) of lambs infected with H. contortus (●——●) and uninfected controls (O----O).

Significance:
Infected < Control ★ $p < 0.01$

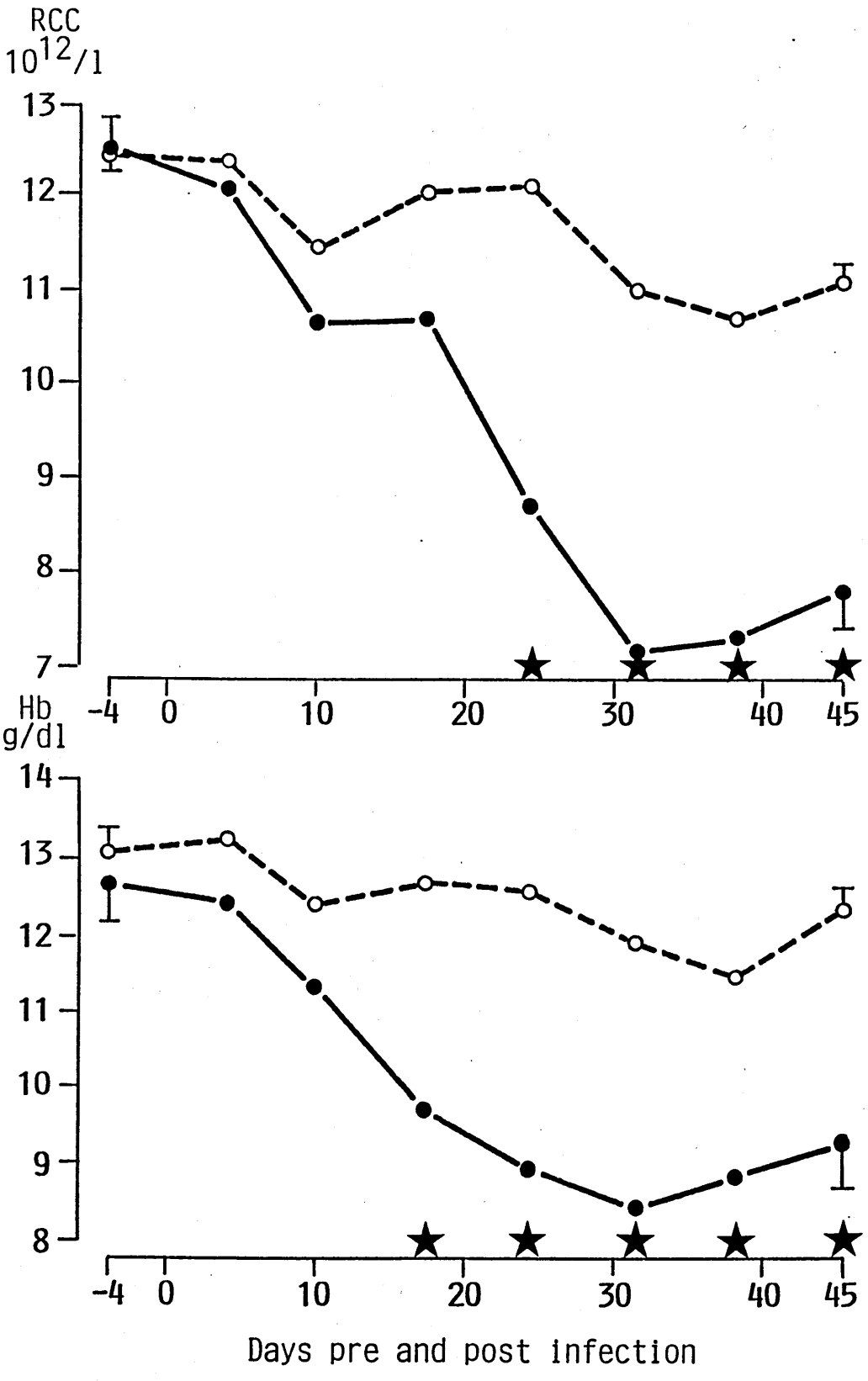

Figure 3a.4 Red cell count (RCC) and haemoglobin (Hb) concentration of lambs infected with H. contortus (●——●) and uninfected controls (○- - -○).

Significance:
Infected < Control ★ $p < 0.01$

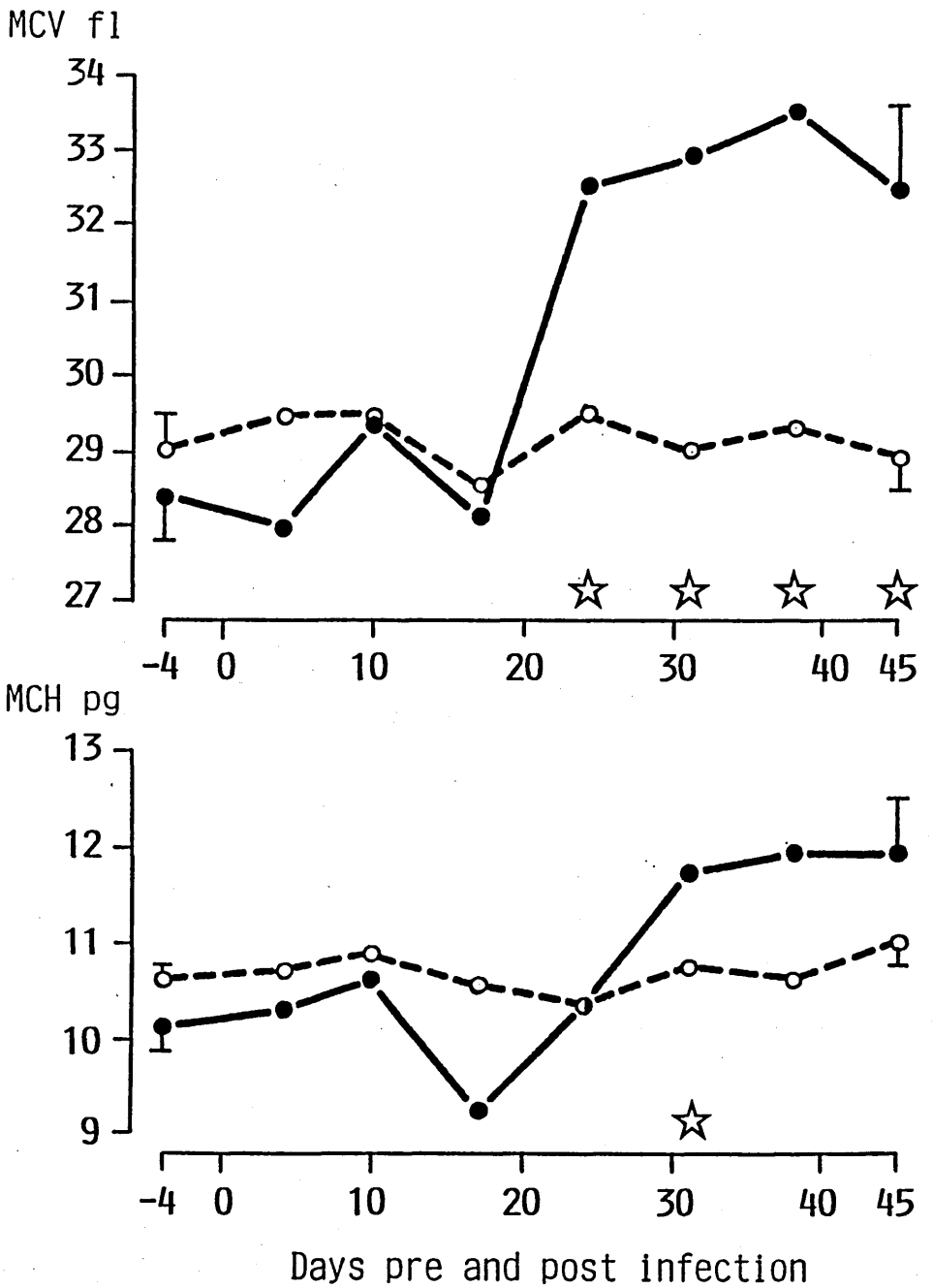

Figure 3a.5 Mean cell volume (MCV) and mean cell haemoglobin (MCH) of lambs infected with H. contortus (●———●) and uninfected controls (O- - -O).

Significance:
Infected > Control ☆ $p < 0.05$

values toward the end of the study (Figure 3a.6).

The mean WCC of the infected sheep fell from a pre-infection value of 11.3 x 10^9/l to 7.1 at 38 DAI (Figure 3a.6). In contrast, values for the control group remained at or above 10 x 10^9/l throughout the experiment.

Biochemical changes

Serum proteins

Infection with H. contortus had no effect on serum albumin, globulin or total protein concentrations which remained around 30, 24 and 54 g/l respectively in each group throughout the experiment (Figure 3a.7).

Serum iron, iron binding capacity and % saturation of transferrin

Mean serum iron, iron binding capacity and percentage saturation of transferrin values varied widely between sampling times in both groups, and overall there was no consistent effect of infection in any of these parameters (Figures 3a.8 & 3a.9).

Plasma glucose

The mean plasma glucose values for each sheep are presented in Table 3a.7. The mean value for the infected sheep (4.36 mmol/l) was significantly higher than those of both control groups (3.94 and 3.92 in groups PFSC and ALC respectively). There was no significant correlation between glucose and insulin values, nor between glucose concentration and PCV in any group.

Endocrine assays

The mean concentrations of insulin, cortisol, growth hormone and prolactin for each group are shown in Table 3a.8 and individual sheep values are presented in Appendix 1 Tables 1 to 4.

The mean insulin values of groups ALSI and ALC were similar at

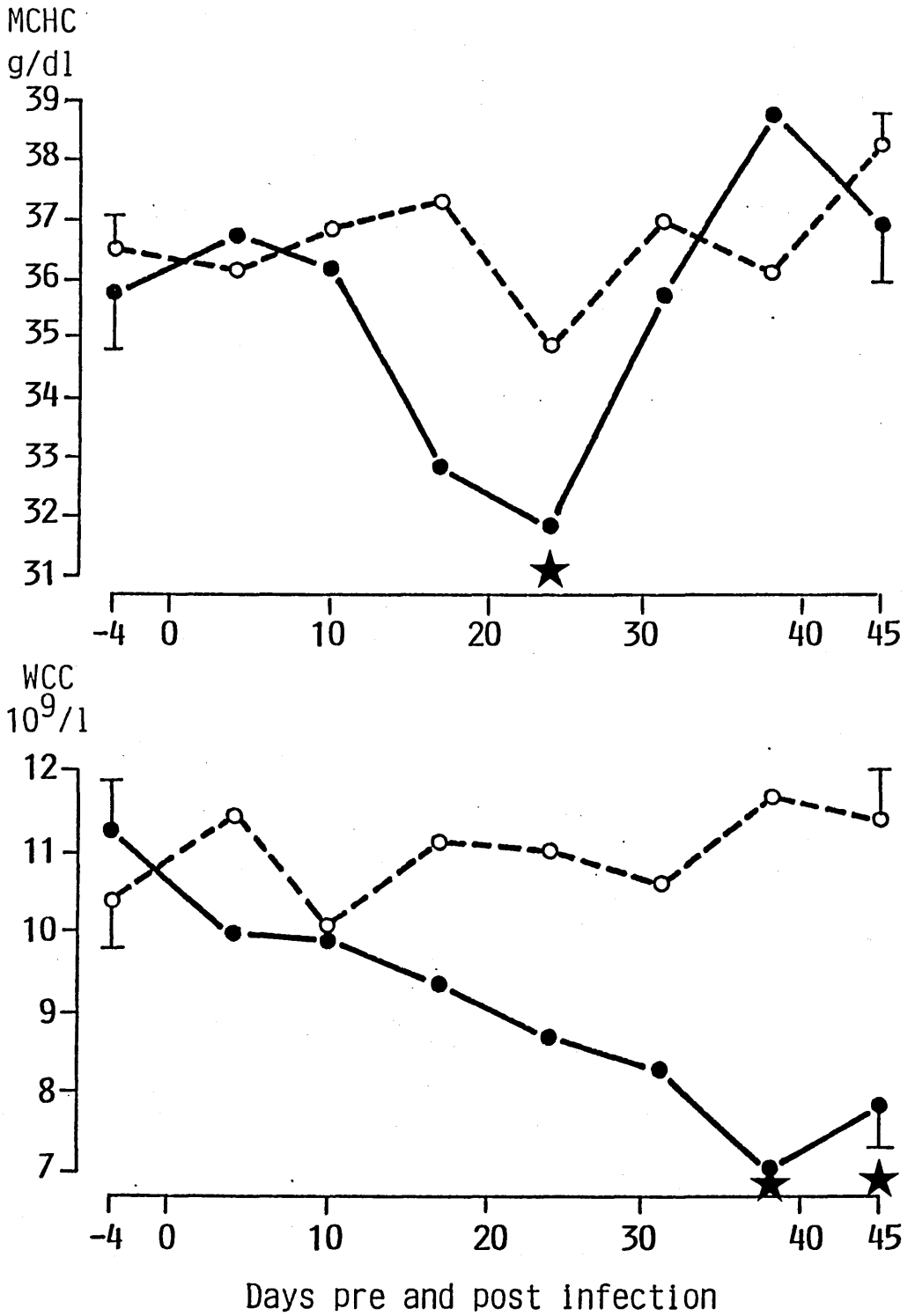

Figure 3a.6 Mean cell haemoglobin concentration (MCHC) and total white cell count (WCC) of lambs infected with H. contortus (●——●) and uninfected controls (O- - - -O).

Significance:
Infected < Control ★ $p < 0.01$

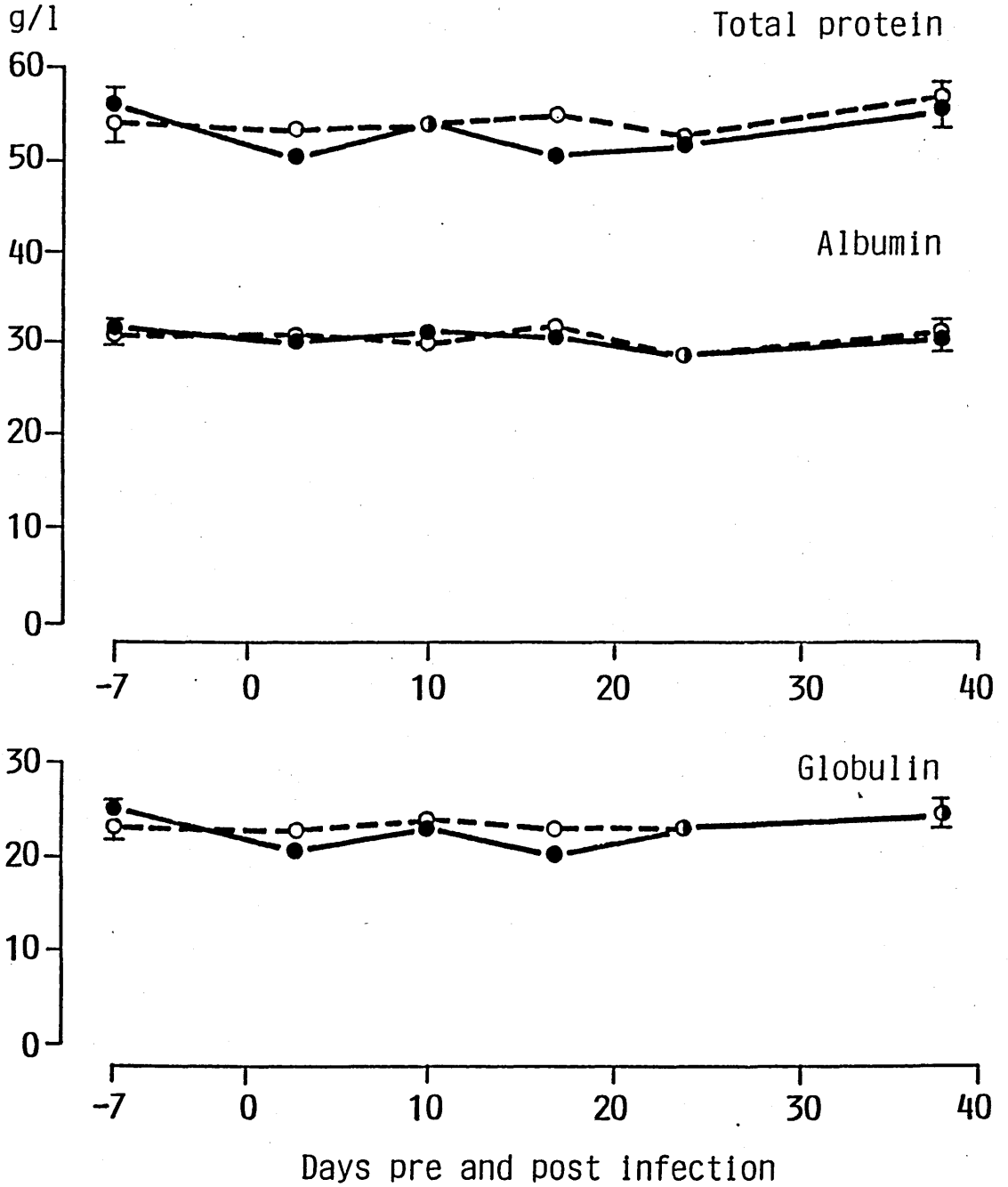

Figure 3a.7 Serum total protein, albumin and globulin concentration of lambs infected with H. contortus (●——●) and uninfected controls (O---O).

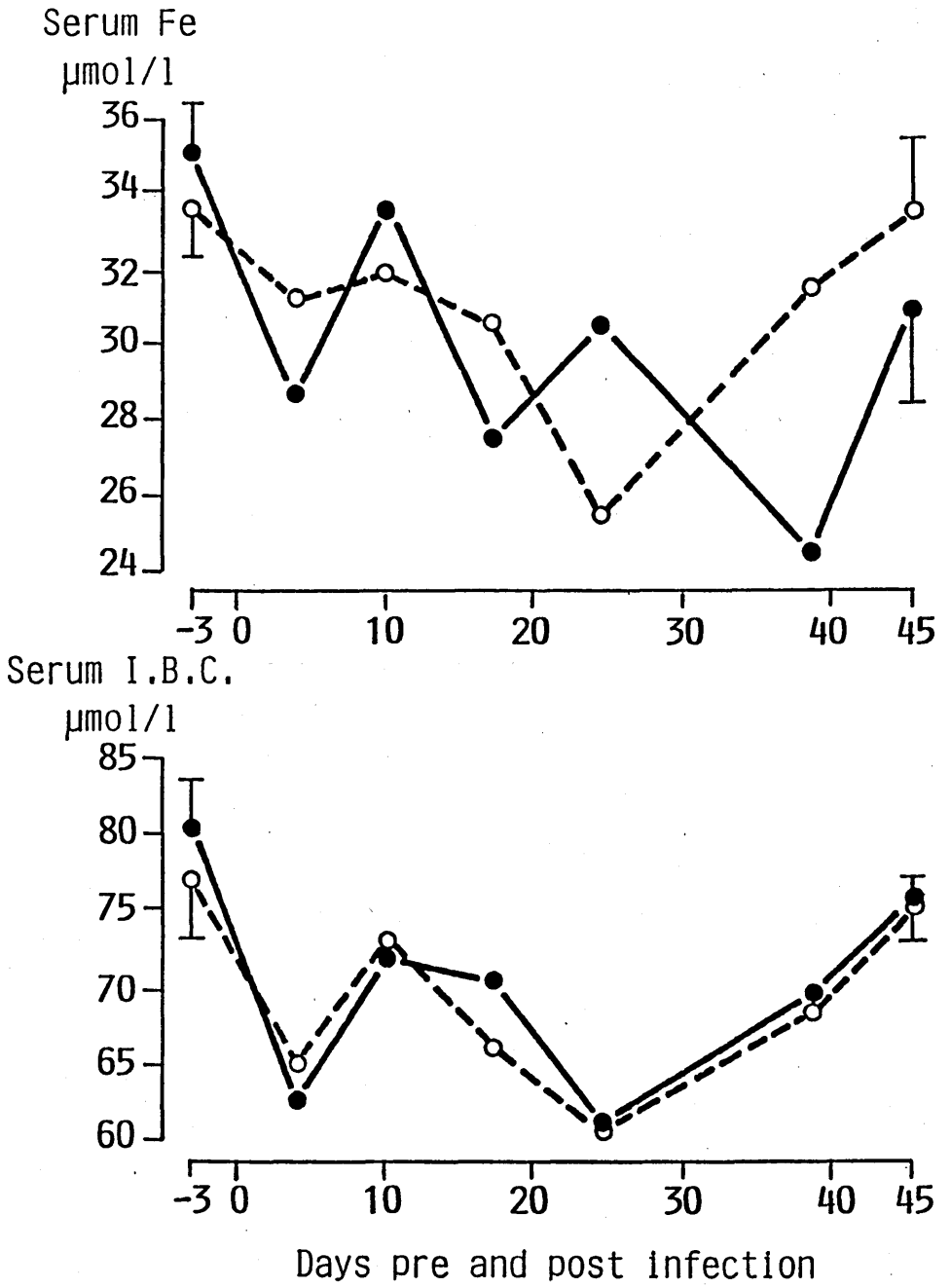

Figure 3a.8 Serum iron (Fe) concentration and iron binding capacity (IBC) of lambs infected with H. contortus (●——●) and uninfected controls (O– – –O).

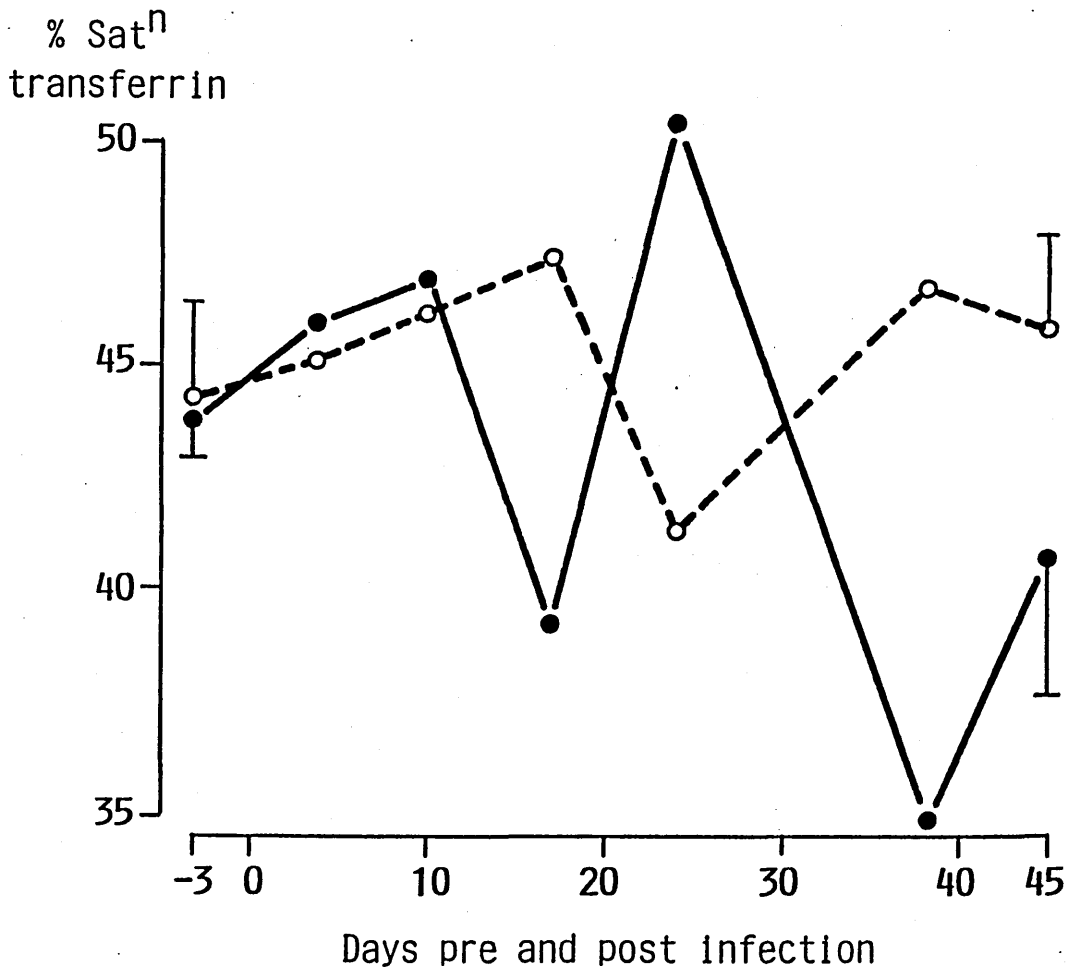

Figure 3a.9 The percentage saturation (% satn) of transferrin of lambs infected with H. contortus (●——●) and uninfected controls (O– – –O).

Table 3 a.7

Plasma glucose concentrations of sheep infected with 350 H. contortus larvae /kg BWt 31 davs previously (ALSI) and uninfected controls pair-fed (PFSC) or offered feed ad libitum (ALC). Figures are mean (± SE) of four samples taken at 8, 9 and 10 a.m. and 12 noon.

Sheep No.	Plasma glucose mmol/l
ALSI-17	4.78 (0.09)
ALSI-31	4.48 (0.09)
ALSI-7	4.35 (0.09)
HLSI-20	4.25 (0.13)
ALSI-27	3.92 (0.13)
PFSC-25	3.68 (0.18)
PFSC-18	3.90 (0.09)
PFSC-12	3.95 (0.09)
PFSC-16	4.02 (0.12)
PFSC-23	4.15 (0.15)
ALC-37	3.78 (0.06)
ALC-41	4.08 (0.14)
ALC-15	4.02 (0.08)
ALC-3	3.98 (0.11)
ALC-19	3.75 (0.12)

Analysis of variance

ALSI > PFSC, ALC $p < 0.05$

Table 3 a.8

Group mean (± S.E.) serum endocrine concentrations (ng/ml) of sheep infected with 350 *H. contortus*/kg BWt 31 days previously (ALSI), and uninfected controls pair-fed (PFSC) or offered feed *ad libitum* (ALC).

Group	Insulin	Growth Hormone	Prolactin	Cortisol
ALSI	0.66 (0.044)	1.54 (0.182)	103.6 (9.03)	10.4 (1.46)
PFSC	0.33 (0.039)	4.57 (1.464)	82.5 (5.88)	11.5 (1.23)
ALC	0.61 (0.061)	1.89 (0.490)	97.8 (7.26)	14.4 (1.12)
Significance	ALSI, ALC > PFSC *	ALSI, ALC > PFSC *	ALSI > PFSC *	ALC > ALSI *

Analysis of variance

* $P < 0.05$

0.66 and 0.61 ng/ml, and both were significantly higher than that of group PFSC (0.33 ng/ml).

The growth hormone concentrations of group ALSI were slightly lower than those of ALC (mean 1.54 and 1.89 ng/ml respectively), and significantly lower than the mean value of group PFSC, 4.57 ng/ml.

Group ALSI had the highest mean prolactin level at 103.6 ng/ml, this being slightly higher than ALC (97.8), and significantly higher than PFSC (82.5 ng/ml).

Considering cortisol concentrations, group ALSI showed the lowest values (mean 10.4 mg/ml), being slightly lower than PFSC (11.5), and significantly lower than ALC (14.4 ng/ml).

The mean values conceal considerable variation within the groups, both between and within individual sheep, as can be seen from Appendix 1 Tables 1 to 4.

Within group ALSI there was a significant positive correlation between the mean insulin concentration and the PCV measured the same day ($r = 0.086$, $p<0.05$). There was no such correlation among the control sheep. Similar analysis of the mean concentrations of the other hormones showed no significant correlations.

3a.3.5 Carcase evaluation

Indicator joint dissection

The group mean weights and composition of the indicator joints are shown in Table 3a.9. There were no significant differences between groups in any of the parameters measured.

Body composition - chemical analysis

The mean body composition of the groups is shown in Table 3a.10. There were no significant differences between groups ALSI, PFSC and ALC in any of the parameters, although the percentage of water in the

Table 3a.9

Mean (± S.E.) weight and composition of the right best end neck joint of sheep infected with H. contortus (ALSI) and of controls pair-fed (PFSC) or offered feed ad libitum (ALC)

Group	Wt. of joint (g)	% Bone	% Fat	% Total Lean	% eye muscle	DM eye muscle
ALSI	436 (35.4)	23.2 (1.38)	30.5 (1.16)	46.2 (1.53)	17.0 (0.63)	0.303 (0.0312)
PFSC	448 (41.7)	19.0 (1.21)	33.2 (1.99)	47.7 (2.57)	17.8 (1.48)	0.292 (0.0240)
ALC	403 (50.4)	24.8 (1.68)	31.1 (3.4)	44.1 (3.11)	17.9 (1.11)	0.328 (0.0426)

Table 3a.10

Body composition of sheep infected with H.contortus (ALSI) 49 days previously, and of controls slaughtered at the commencement of the experiment (IC), pair-fed (PFSC) or offered food ad libitum (ALC)

Group n	IC 5	ALSI 5	PFSC 5	ALC 5
Body Wt (kg)	23.9 (3.60)	35.7 (2.58)	37.1 (2.40)	34.2 (2.04)
Empty body wt (kg)	19.6 (3.23)	29.4 (2.06)	28.6 (1.83)	27.6 (1.71)
Total body fat (kg)	3.37 (0.962)	6.23 (0.507)	6.60 (0.771)	6.20 (0.806)
Body water (% in fat-free empty body)	74.5 (0.39)	72.5 (0.41)	74.2 (0.65)	73.3 (0.50)
Body protein (% in fat-free empty body)	19.7 (0.26)	19.6 (0.26)	20.6 (0.28)	19.7 (0.24)
Total body protein (kg)	3.21 (0.494)	4.53 (0.284)	4.55 (0.284)	4.23 (0.228)

fat-free body tended to be lower ($p<0.1$) among group ALSI (72.5 %) than the controls (74.2 and 73.3 % in groups PFSC and ALC). The composition of the net empty bodyweight gain over the course of the experiment is shown in Table 3a.11. The mean gain among group ALSI contained less fat (300 g/kg) than groups PFSC and ALC (377 and 344 g/kg respectively) but the difference was not significant. The difference in fat content was reflected in a small difference in energy values, while the protein content of the gain was similar in each group at 126 - 146 g/kg.

Percentage retention of dietary gross energy and crude protein

The percentage retention of dietary GE and CP are shown in Table 3a.12. Infected sheep tended to retain a higher percentage of both GE and protein, although the differences were not significant.

Prediction of body composition from indicator joint analysis

Equations were formulated by regressing, for 15 sheep, the percentage fat in the indicator joint with the percentage fat in the carcase and whole body, and the percentage lean meat in the indicator join with the percentage protein in the carcase and whole body. The regressions (Table 3a.13) were all significant. The predictions of fat content were more reliable than those of protein. For both fat and protein, the indicator joint gave a better prediction of the composition of the carcase than of the whole body. Because of the lack of effect of infection on these parameters, it did not prove profitable to test differences between groups in regressions of indicator joint against whole body composition.

Table 3a.11

Composition of 1 kg net gain in empty bodyweight of sheep infected with *H. contortus* for 49 days (ALSI) and of controls pair-fed (PFSC) or offered food *ad libitum* (ALC)

	Fat (g)	Protein (g)	Energy (MJ)
ALSI	300 (30.2)	134 (7.0)	17.0 (1.40)
PFSC	377 (40.3)	146 (5.1)	17.8 (0.46)
ALC	344 (37.7)	126 (4.7)	17.3 (1.45)

Table 3a.12

The % retention of dietary GE and CP by lambs infected with 350 *H. contortus*/kg BWt (ALSI) and of controls pair-fed (PFSC) or offered feed *ad libitum* (ALC).

Group	% Retention of dietary GE	% Retention of dietary CP
ALSI	12.0 (1.44)	12.3 (1.45)
PFSC	10.7 (1.17)	11.3 (1.14)
ALC	11.4 (1.01)	10.9 (1.04)

Table 3a.13

Equations of the form $Y = (b \pm \text{s.e. of } b)X + A$ from regressions of indicator joint analysis and body or carcase composition. $n = 15$ in each case.

$Y = \%\ EE_{(carcase)}$	X = % fat (joint)	$Y = (0.525 \pm 0.102)X + 10.39$	$t = 5.15$	$P < 0.001$
$Y = \%\ EE_{(body)}$	X = % fat (joint)	$Y = (0.417 \pm 0.091)X + 8.83$	$t = 4.58$	$P < 0.001$
$Y = \%\ protein_{(carcase)}$	X = % lean (joint)	$Y = (0.108 \pm 0.026)X + 9.89$	$t = 4.15$	$P < 0.002$
$Y = \%\ protein_{(body)}$	X = % lean (joint)	$Y = (0.051 \pm 0.018)X + 13.2$	$t = 2.83$	$P < 0.02$

Experiment 3b The effects of repeated infection with small numbers of *H. contortus* on the growth, body composition and certain metabolic parameters of lambs offered feed *ad libitum*.

3b.2 Materials and methods

3b.2.1 Experimental design

Thirty-five lambs from the same rearing group as those used in the previous experiment, and including 10 common to it, were used to investigate the effect of a trickle infection with *H. contortus* larvae on production in growing lambs. The lambs were allocated to experimental groups on the basis of Hb type and bodyweight.

Five lambs, group IC, were killed at the start of the experiment. These were common to Experiment 3a.

Group ALI comprised 10 lambs given 20 *H. contortus* larvae/kg BWt twice weekly throughout the experiment. 5 were killed after 53 days of infection, the other 5 on day 109. The lambs of group ALI were offered feed individually to appetite.

Group PFC was 10 lambs, uninfected and pair-fed to individuals of group ALI. 5 of these lambs were killed on day 60 of the experiment, the other 5 on day 109. The former five lambs were pair-fed exactly as in Experiment 3a, ie. on a weekly basis when in pens and on a daily basis while in metabolism stalls, with a one week delay throughout. The only difference with the latter five lambs was that their nutritional balance study was conducted concurrently with their respective infected partners, with a one day lag in pair-feeding during this period. They were killed on the same day as their infected partners.

Group ALC comprised 10 uninfected lambs fed to appetite. Five killed on day 53 were common to Experiment 3a. The other five were killed on day 109.

The allocation of individual lambs to groups, and their Hb types, are shown in Tables 3a.1 and 3b.1.

Haematological and serum protein and iron changes, faecal egg counts and body weights were measured weekly throughout the study. Nutritional balance studies were conducted one week prior to slaughter on the sheep of groups ALI and PFC, and on those of group ALC which were killed on day 109. At slaughter, abomasal worm burdens were assessed, and carcase analysis was carried out as described for Experiment 3a. The accomodation, feed and feeding arrangements were the same as those described for Experiment 3a.

3b.2.2 Experimental infections

Sheep of group ALI were infected twice weekly with oral doses of approximately 20 infective H. contortus larvae/kg BWt, based on the group mean bodyweight. For the first 5 weeks each dose comprised 500 larvae. This was then increased to 750 for 5 weeks, and from week 10 to 15 each lamb recieved 1,000 larvae twice weekly.

3b.3 Results

3b.3.1 Clinical and bodyweight changes

With one exception (ALI-38, see below), the sheep remained clinically healthy throughout the experiment, and grew well except when they were moved to metabolism stalls where they initially tended to lose some weight (Figure 3b.1). Over the first 50 days of infection, the sheep of ALI grew at an average rate of 221 g/day, while groups PFC and ALC achieved daily liveweight gains (DLG) of 209 and 224 g respectively. Sheep ALI-8 was the smallest and

Table 3b.1

The Haemoglobin (Hb) types of lambs given repeated doses of small numbers of *H. contortus* larvae (ALI) and control sheep pair-fed (PFC) or offered food *ad libitum* (ALC).

Group ALI		Group PFC		Group ALC		
Sheep No.	Hb type	Sheep No.	Hb type	Sheep No.	Hb type	
ALI-8	B	PFC-30	B	ALC-37	B	
ALI-14	AB	PFC-24	AB	ALC-41	B	
ALI-22	B	PFC-21	AB	ALC-15	AB	Killed 53 DAI
ALI-35	B	PFC-46	B	ALC-3	AB	
ALI-1	AB	PFC-9	B	ALC-19	B	
ALI-10	B	PFC-36	B	ALC-29	B	
ALI-43	AB	PFC-40	B	ALC-32	B	
ALI-33	AB	PFC-34	AB	ALC-4	AB	Killed 109 DAI
ALI-28	AB	PFC-2	AB	ALC-39	AB	
ALI-38	B	PFC-44	AB	ALC-42	B	

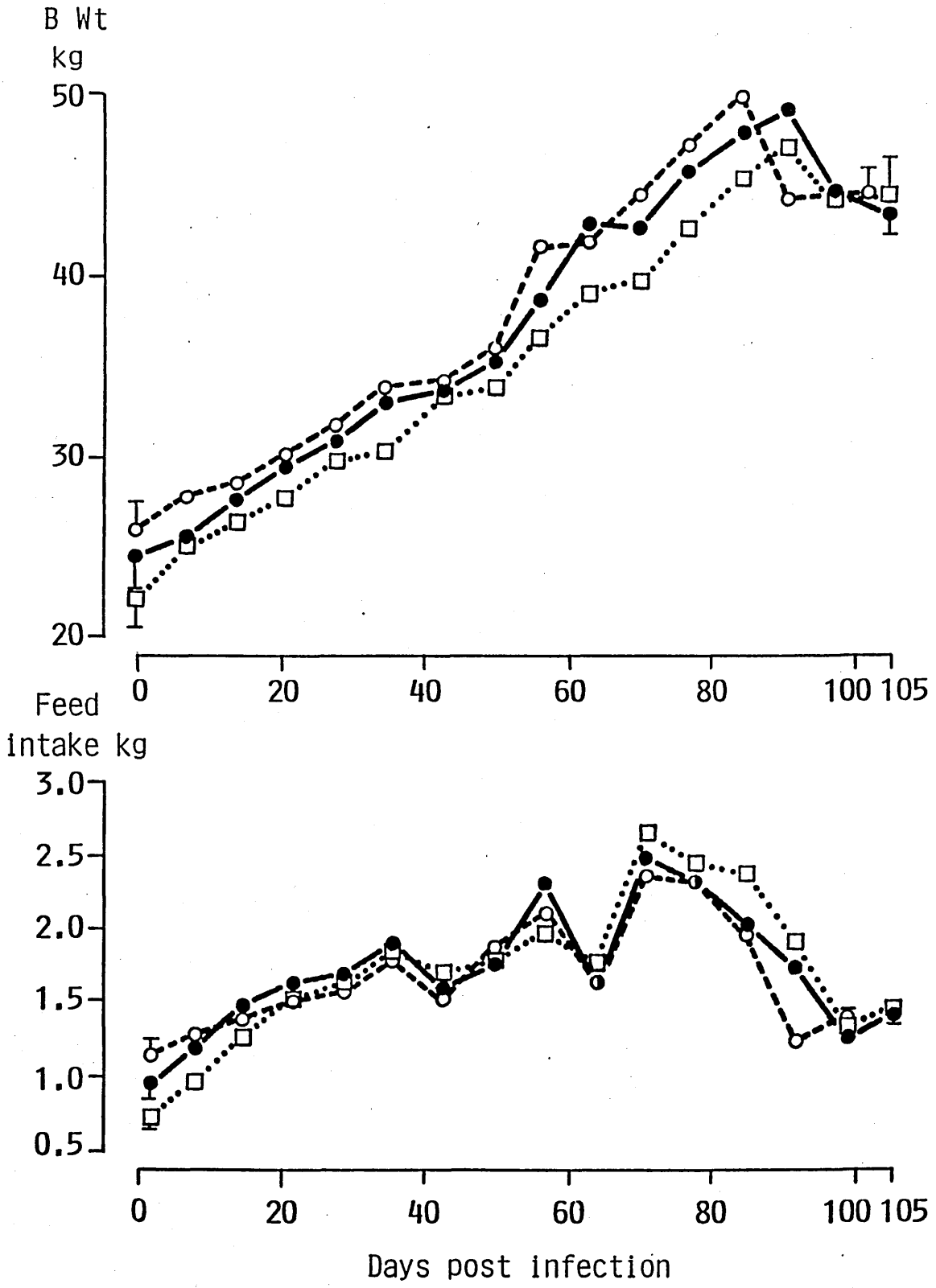

Fig. 3b.1 Bodyweight (BWt) and daily feed intake of lambs given a trickle infection of H. contortus (●——●) and controls pair-fed (O- - -O) or offered feed ad libitum (□·····□).

slowest-growing infected sheep, and it and its pair-fed control only achieved DLG of 70 and 92 g, compared to 150 g by ALC-29, the smallest sheep of group ALC. During the second half of the experiment, mean DLG of the surviving sheep were 100, 133 and 115 g among groups ALI, PFC and ALC respectively. The differences between the groups were not significant at any stage of the infection.

Around 84 DAI, ALI-38 became obviously unwell. The sheep was found in a sitting-dog position, and was dull, tachypnoeic, anorexic and grinding its teeth. It had a rectal temperature of 39.5^{o}C, a PCV of 0.25 l/l (compared to 0.32 the previous week), and its blood copper concentration was 21.5 umol/l (ie. within normal limits). It was apparent that the sheep was suffering from anterior abdominal pain, but no firm diagnosis was reached and no treatment was given. Over the next week the sheep lost 4 kg bodyweight, but its appetite and clinical condition improved and the sheep made an almost complete recovery. At slaughter on day 109, the sheep was found to have a hepatic abscess with adhesions to the reticular wall. No foreign body was located, but a retrospective diagnosis of a penetrating reticular foreign body was made.

3b.3.2 Parasitological findings

Faecal egg counts

Strongyle eggs were present in the faeces of all the infected sheep by 21 DAI, and the mean epg peaked at 7,275 at 49 DAI (Figure 3b.2). There was considerable variation in the individual egg counts, and in the pattern of egg excretion. ALI-8 showed the highest epg (25,300 at 35 DAI). The sheep killed at 53 DAI were excreting substantial numbers of eggs at the time of slaughter (mean 8.8 x 10^{6} eggs per day, range 3.6 to 12.7 x 10^{6}). By day 106,

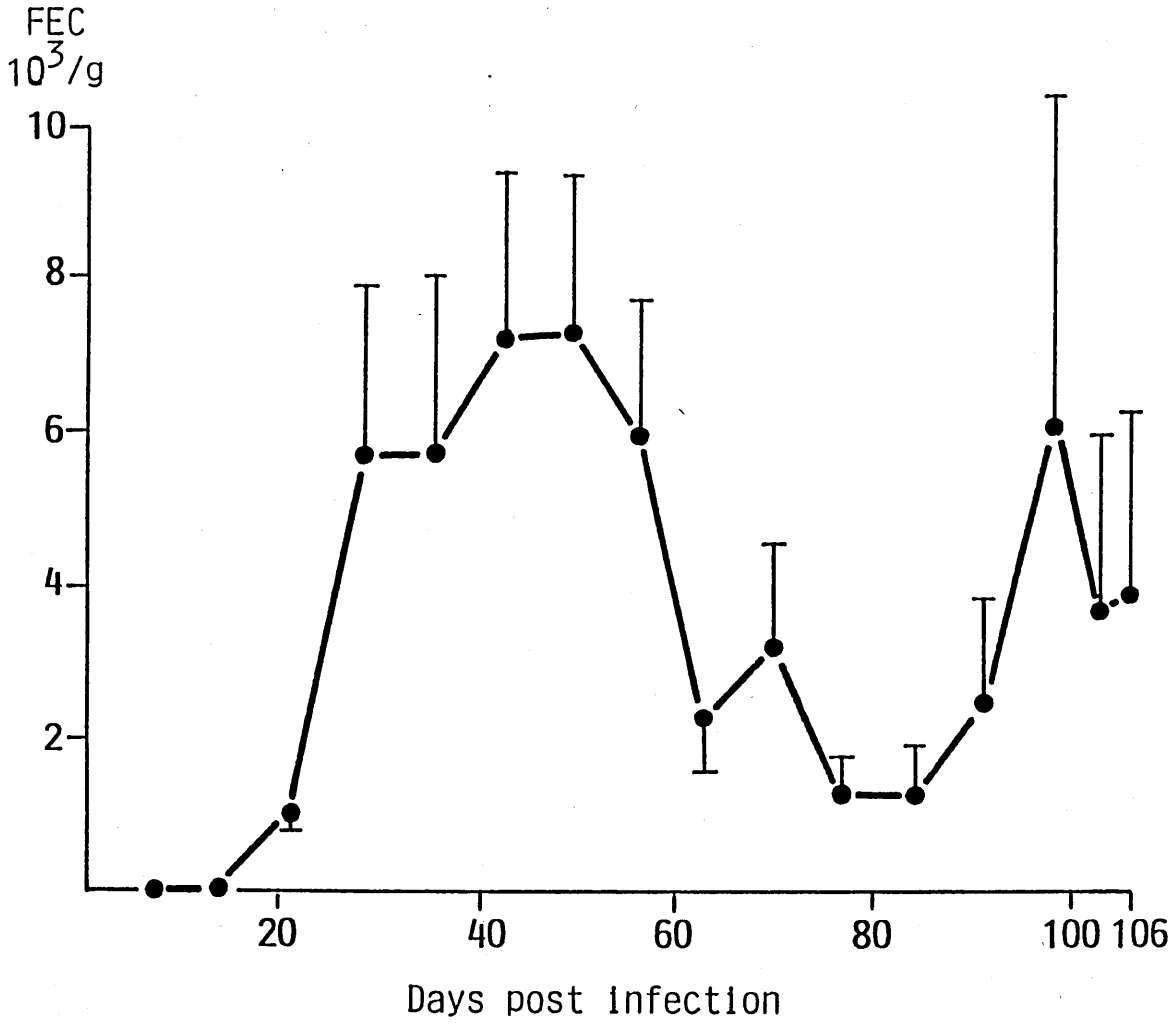

Figure 3b.2 Faecal egg count (FEC) of lambs given a trickle infection of H. contortus (●——●).

the egg outputs of the remaining sheep were lower, but more variable (mean 3.6 x 10^6, range 0.03 to 11.3 x 10^6). The data for the individual sheep are shown in Table 3b.2.

Worm burdens

The mean number of worms recovered from the sheep killed 53 and 109 DAI were 1,874 and 1,434 respectively, with male/female ratios of 1.02 and 1.04, and the percentage of the recovered population which were immature being 20 and 27 % respectively. Individual sheep data are shown in Table 3b.2. For the sheep killed 53 DAI, the recovered worms represented 20 % of the original larval challenge, whilst from those killed 109 DAI, 6 % of the total larvae administered were recovered.

There was a significant positive correlation between the number of worms recovered at slaughter and the total daily egg output a few days earlier among the sheep killed at day 109 ($p<0.05$), but not among those killed at day 53.

Control sheep

One control sheep, PFC-2, had 2,800 epg faeces on day 98, and 570 adult and immature H. contortus were recovered from its abomasum at slaughter. None of the other control sheep excreted nematode eggs at any time, nor were any worms found in their abomasa.

3b.3.3 Nutritional studies

Feed Intake

Intake by all the sheep increased over the course of the experiment, except when they were placed in metabolism stalls when a marked drop in intake was observed in most of the sheep (Figure 3b.1). There were no differences between the groups in feed intake at any stage of the infection. Sheep ALI-8, the smallest infected

Table 3b.2

Individual worm burdens and total daily egg outputs of lambs given repeated infections of small numbers of H. contortus larvae.

Sheep No.	Killed DAI	Total larval challenge	Worms recovered at slaughter				Larval Stages % of Recovered Worms	Total Worms % of Larval Challenge	Total egg output/day 6 days prior to slaughter
			Larval Stages	Adult Male	Adult Female	Total			
ALI-8	53	8,750	890	1500	1260	3650	24	42	7,548,000
ALI-14	53	8,750	380	550	800	1730	22	20	9,634,000
ALI-22	53	8,750	360	300	370	1030	35	12	10,516,000
ALI-35	53	8,750	70	160	140	370	19	4	3,614,000
ALI-1	53	8,750	110	990	870	1970	6	23	12,747,000
ALI-10	109	23,500	430	750	570	1750	25	7	4,437,000
ALI-43	109	23,500	670	510	570	1750	38	7	1,587,000
ALI-33	109	23,500	10	20	40	70	14	0.3	32,000
ALI-28	109	23,500	800	1200	1220	3220	25	14	11,302,000
ALI-38	109	23,500	60	150	140	350	17	1	644,000

sheep, ate much less than the other members of its group. Its mean daily intake was 0.52 kg, with the mean intakes of the other infected sheep ranging from 1.35 to 2.00 kg. Because of the low intake by ALI-8, a modified pair-feeding arrangement was adopted whereby its partner, PFC-30, was offered a minimum of 0.60 kg/day, or more if ALI-8 had consumed more. The mean intake of PFC-30 was 0.64 kg/day.

As mentioned above, ALI-38 was anorexic during the period of its traumatic reticulitis, after which its feed intake recovered to almost its previous level.

Digestibility trial

The group mean digestibility coefficients are shown in Table 3b.3. There were no significant differences between the groups within each study, or between the first and second studies, with the exception that CP digestibility was significantly higher in all the sheep at the second study. Sheep ALI-8 and its partner PFC-30 had higher coefficients of digestion of DM, OM, CP and GE than the other sheep of their groups.

Nitrogen balance

All the sheep were in positive N balance (Table 3b.4). At the first balance study, N retention of the infected sheep was significantly lower than that of the controls (4.7 and 7.0 g/day respectively). At the second study there were no differences between the groups, with mean values of 6.3, 6.4 and 6.9 g/day in groups ALI, PFC and ALC.

Water balance

The mean amount of water apparently 'retained' by the sheep 40 - 47 DAI was 1.5 l/day in both groups ALI and PFC (Table 3b.4). Water balance was not measured at the second study period.

Table 3b.3

Digestibility coefficients of lambs infected repeatedly with small numbers of H. contortus larvae (ALI), and control lambs pair-fed (PFC) or offered food ad libitum (ALC).

DAI	Group	DM	CP	CF	EE	Ash	NFE	OM	CE
43-50	ALI n = 5	0.637 (0.017)	0.522 (0.021)	0.306 (0.052)	0.821 (0.026)	0.429 (0.015)	0.764 (0.019)	0.661 (0.020)	0.616 (0.024)
	PFC n = 5	0.642 (0.013)	0.558 (0.014)	0.226 (0.039)	0.830 (0.019)	0.452 (0.013)	0.780 (0.008)	0.663 (0.013)	0.619 (0.014)
	ALI n = 5	0.637 (0.012)	0.589 (0.008)	0.220 (0.028)	0.787 (0.020)	0.407 (0.038)	0.775 (0.009)	0.661 (0.010)	0.639 (0.011)
99-106	PFC n = 5	0.654 (0.008)	0.599 (0.014)	0.265 (0.028)	0.792 (0.011)	0.434 (0.027)	0.786 (0.005)	0.677 (0.007)	0.650 (0.008)
	ALC n = 5	0.635 (0.006)	0.593 (0.010)	0.212 (0.011)	0.791 (0.028)	0.444 (0.021)	0.765 (0.009)	0.655 (0.006)	0.631 (0.011)
Significance			**						

** All sheep (43-50 DAI) < All sheep (99-106 DAI); $p<0.001$

Table 3b.4

Nitrogen and water balances of sheep infected with H. contortus (ALI) and uninfected controls pair-fed (PFC) or offered feed ad libitum (ALC).

DAI	40 - 47				99 - 106		
	N balance g/day		Water balance l/day		N balance g/day		
	ALI	PFC	ALI	PFC	ALI	PFC	ALC
n	5	5	5	5	5	5	5
Intake	27.4 (3.16)	28.1 (3.14)	3.3 (0.45)	3.6 (0.41)	27.3 (1.10)	26.5 (1.09)	28.1 (0.72)
Faeces	13.3 (1.85)	12.6 (1.66)	0.9 (0.17)	0.9 (0.15)	11.2 (0.61)	9.9 (0.77)	11.4 (0.21)
Urine	9.4 (1.63)	8.5 (1.11)	0.8 (0.07)	1.2 (0.13)	9.8 (1.34)	9.5 (1.68)	9.8 (0.93)
'Retained'	4.7* (1.15)	7.0* (1.00)	1.5 (0.25)	1.5 (0.21)	6.3 (1.57)	6.4 (2.13)	6.9 (0.70)

Paired t-test:

* ALI < PFC, $P < 0.05$

3b.3.4 Haematological changes

The infected sheep developed a mild to moderate, macrocytic, normochromic anaemia during the first 49 days of the experiment, and then recovered toward the end of the experiment.

The mean PCV of group ALI decreased from a pre-infection value of 0.355 l/l to 0.275 by 42 DAI (Figure 3b.3). Thereafter it recovered gradually and had returned to pre-infection levels by the end of the experiment. The mean value of the control sheep remained above 0.307, and was significantly higher than that of the infected group from 21 to 49 DAI.

Haemoglobin and RCC changes (Figure 3b.4) largely paralled the changes in PCV. Hb concentrations fell slightly among group ALI from a mean of 12.8 g/dl before infection to 10.2 by 49 DAI and thereafter returned to almost the pre-infection value. Hb values among the control sheep showed little change, and were significantly higher than the infected sheep from 21 to 49 DAI. The mean RCC of group ALI fell from a preinfection value of 12.96 x 10^{12}/l to 8.97 by 49 DAI. RCC values of the remaining infected sheep recovered to around 12 x 10^{12}/l by 105 DAI. Control sheep showed little change in RCC, and the mean value of the infected sheep was significantly lower than that of the controls from 35 to 56 DAI.

There was considerable variation in the effect of infection on haematological parameters in the individual sheep of group ALI. ALI-8 developed the lowest PCV (0.20 l/l), RCC (5.98 x 10^{12}/l) and Hb (7.2 g/dl) within the group. ALI-1, 10, 22, 33 and 35 also showed marked depressions in these indices, while the remaining sheep of the group showed small or no such effects of infection.

The mean MCV value of group ALI rose slightly from 28.7 fl to

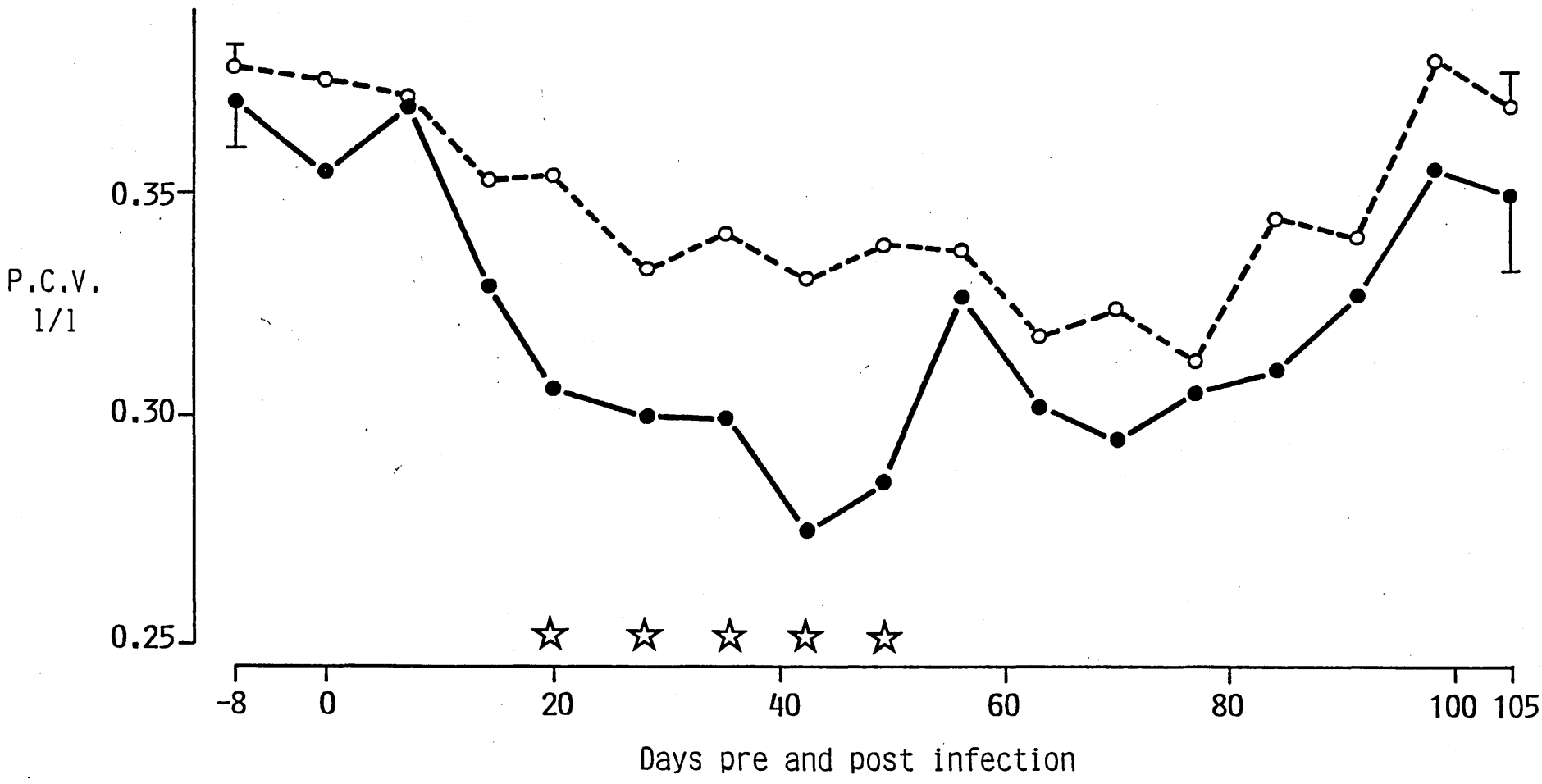

Figure 3b.3 Packed cell volume (PCV) of lambs given a trickle infection of H. contortus (●———●) and uninfected controls (O– – –O).

Significance:
Infected < Control ☆ $p < 0.05$

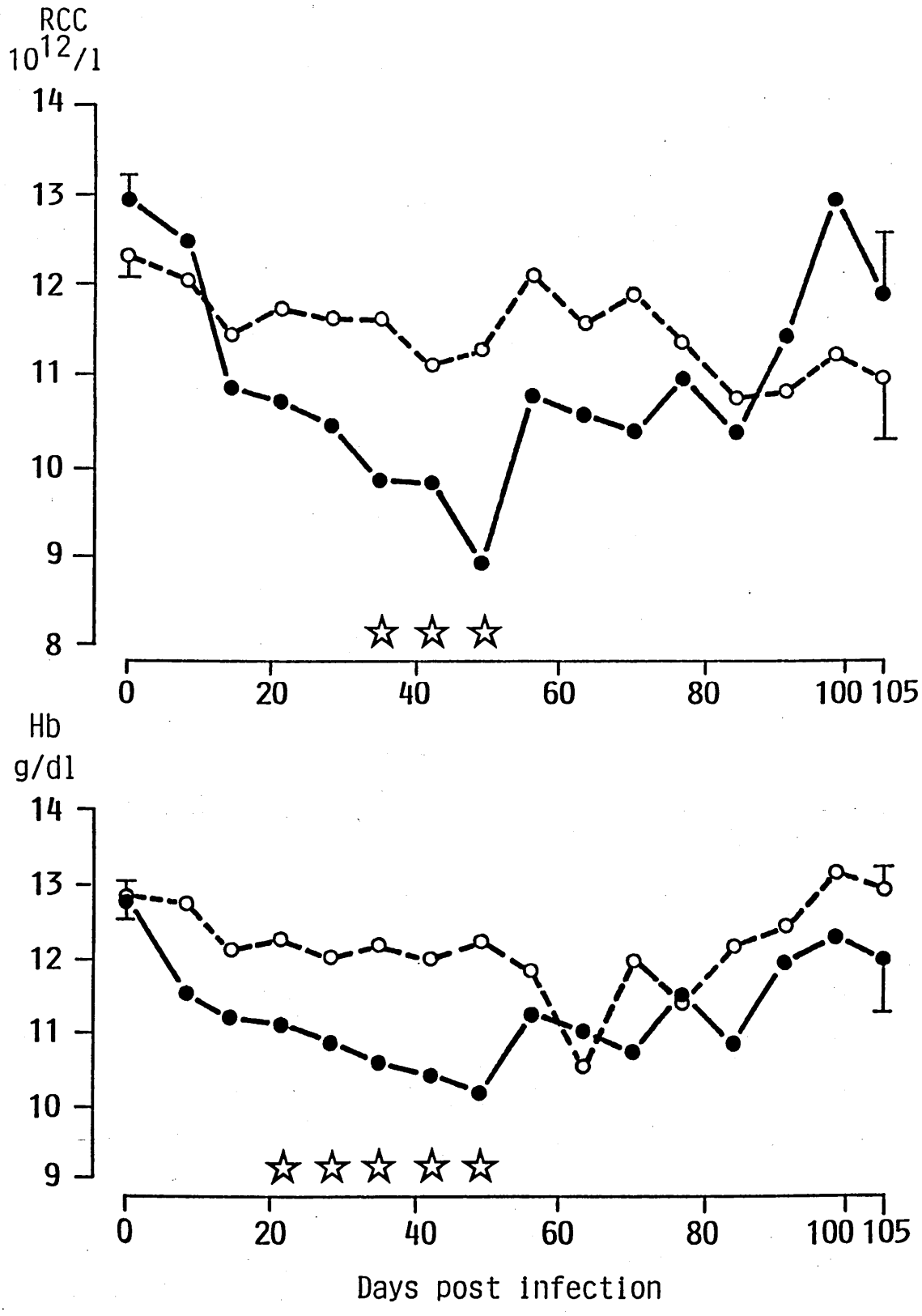

Figure 3b.4 Red cell count (RCC) and haemoglobin (Hb) concentration of lambs given a trickle infection of H. contortus (●——●) and uninfected controls (O---O).

Significance:
Infected < Control ☆ $p < 0.05$

31.0 at 70 DAI before returning to the pre-infection level, and was significantly higher than that of the control group at 49 and 56 DAI (Figure 3b.5). The control group then showed a rise in MCV to 31.2 fl, and thereafter values for all the sheep fell to preinfection levels.

Mean cell haemoglobin (MCH) values in group ALI rose from 9.9 to 11.3 pg by 49 DAI, and then returned to preinfection levels. The control sheep showed less change, but at no time was the difference between the groups significant (Figure 3b.5). MCHC values varied throughout the experiment, but no differences were apparent between the groups (Figure 3b.6).

Infected sheep generally had lower WCCs than the controls during the experiment, with a drop in counts occuring in both groups around 60 DAI.

3b.3.5 Biochemical changes

Serum proteins

Infection had little effect on serum globulin, albumin or total protein concentrations which were around 25, 30 and 55 g/l respectively (Figure 3b.7).

Serum iron, iron binding capacity and the % saturation of transferrin

Although the figures for these parameters showed some variation over the course of the experiment there was no consistent effect due to infection (Figure 3b.8 and 3b.9).

3b.3.6 Carcase evaluation

Indicator joint dissection

Infected sheep tended to have more bone, less fat and slightly more lean than control sheep, although the differences were not

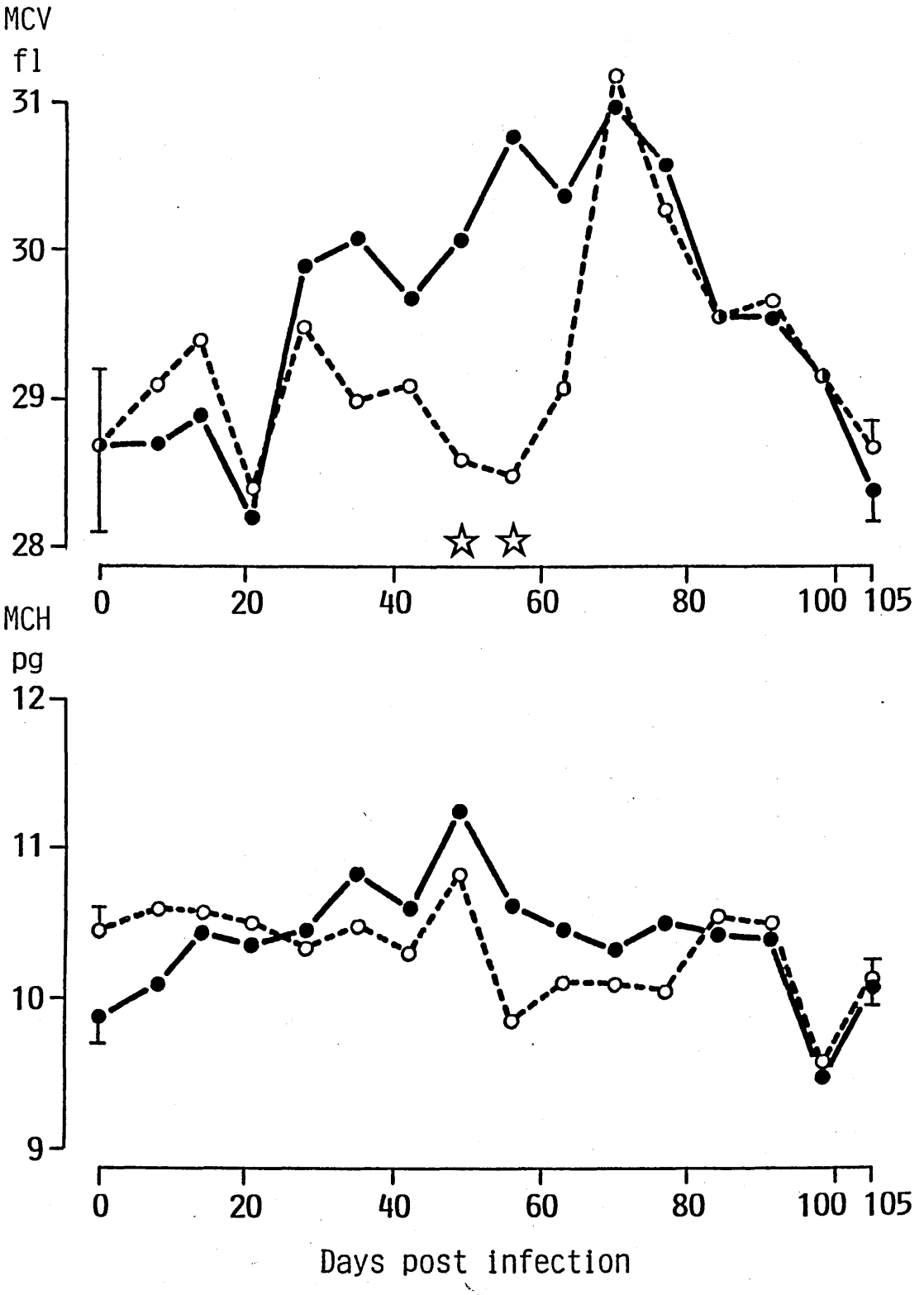

Figure 3b.5 Mean cell volume (MCV) and mean cell haemoglobin (MCH) of lambs given a trickle infection of H. contortus (●——●) and uninfected controls (O- - - -O).

Significance:
Infected > Control ☆ $p < 0.05$

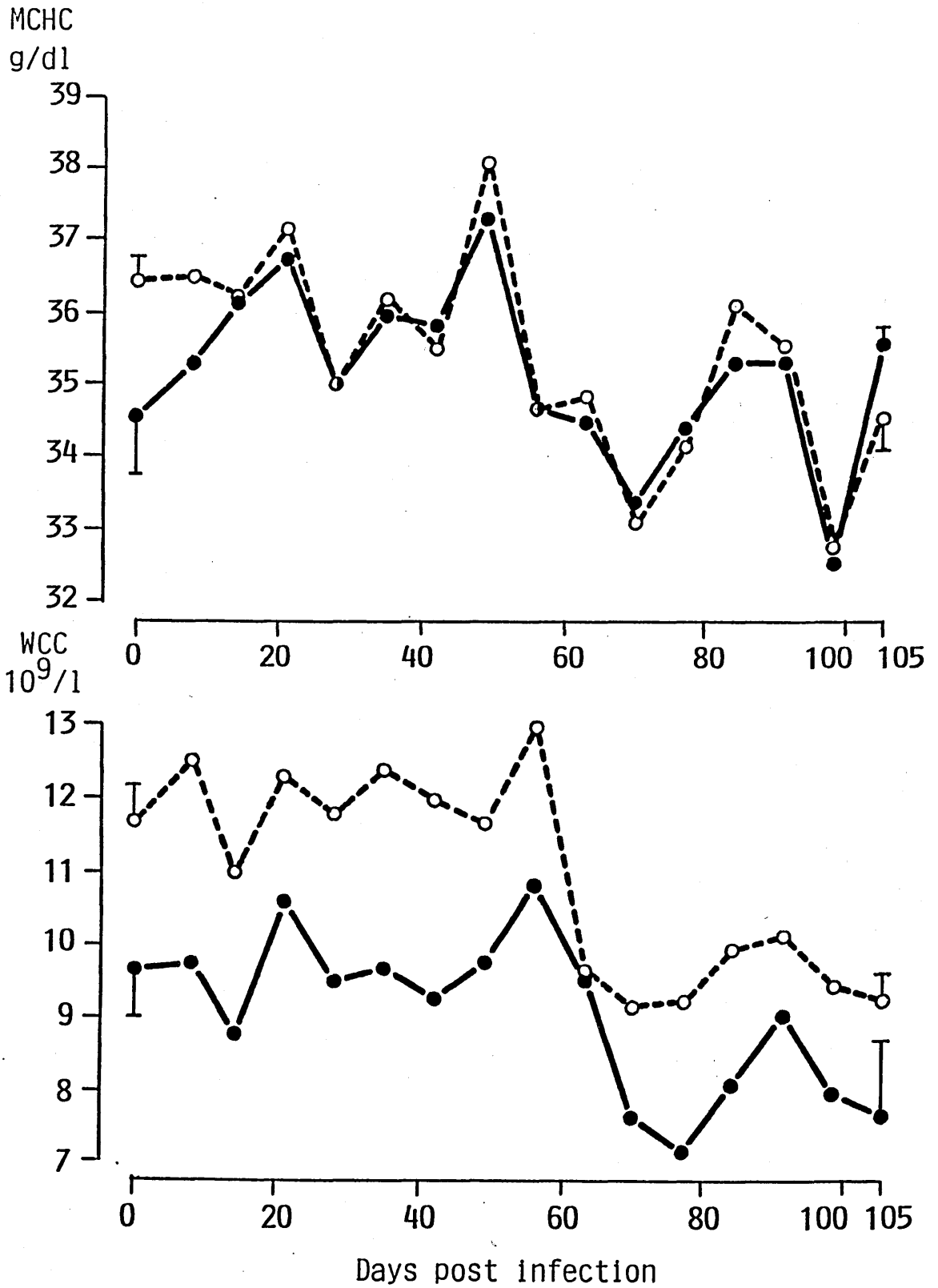

Figure 3b.6 Mean cell haemoglobin concentration (MCHC) and total white cell count (WCC) of lambs given a trickle infection of H. contortus (●——●) and uninfected controls (O– – –O).

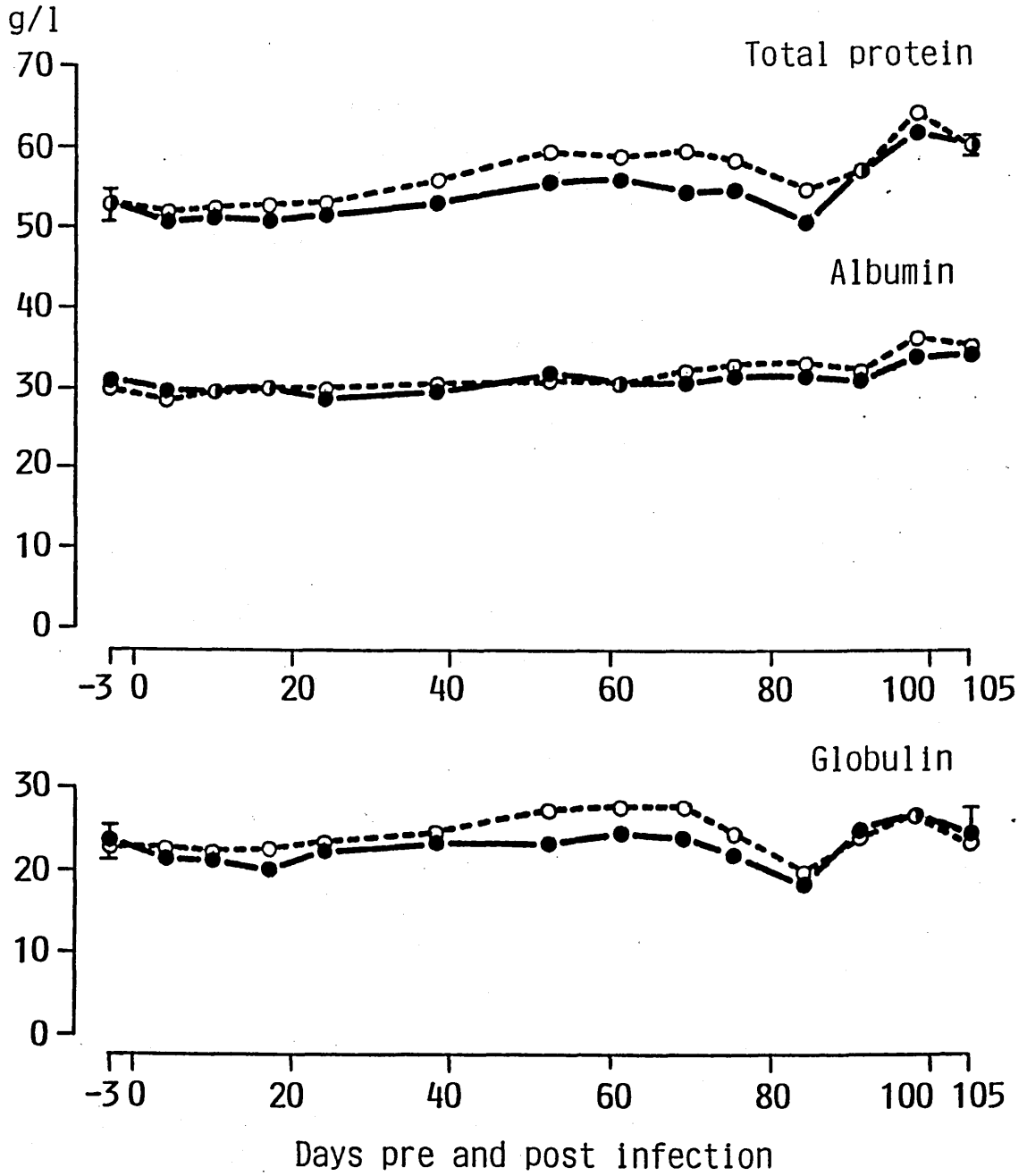

Figure 3b.7 Serum total protein, albumin and globulin concentration of lambs given a trickle infection of H. contortus (●——●) and uninfected controls (O---O).

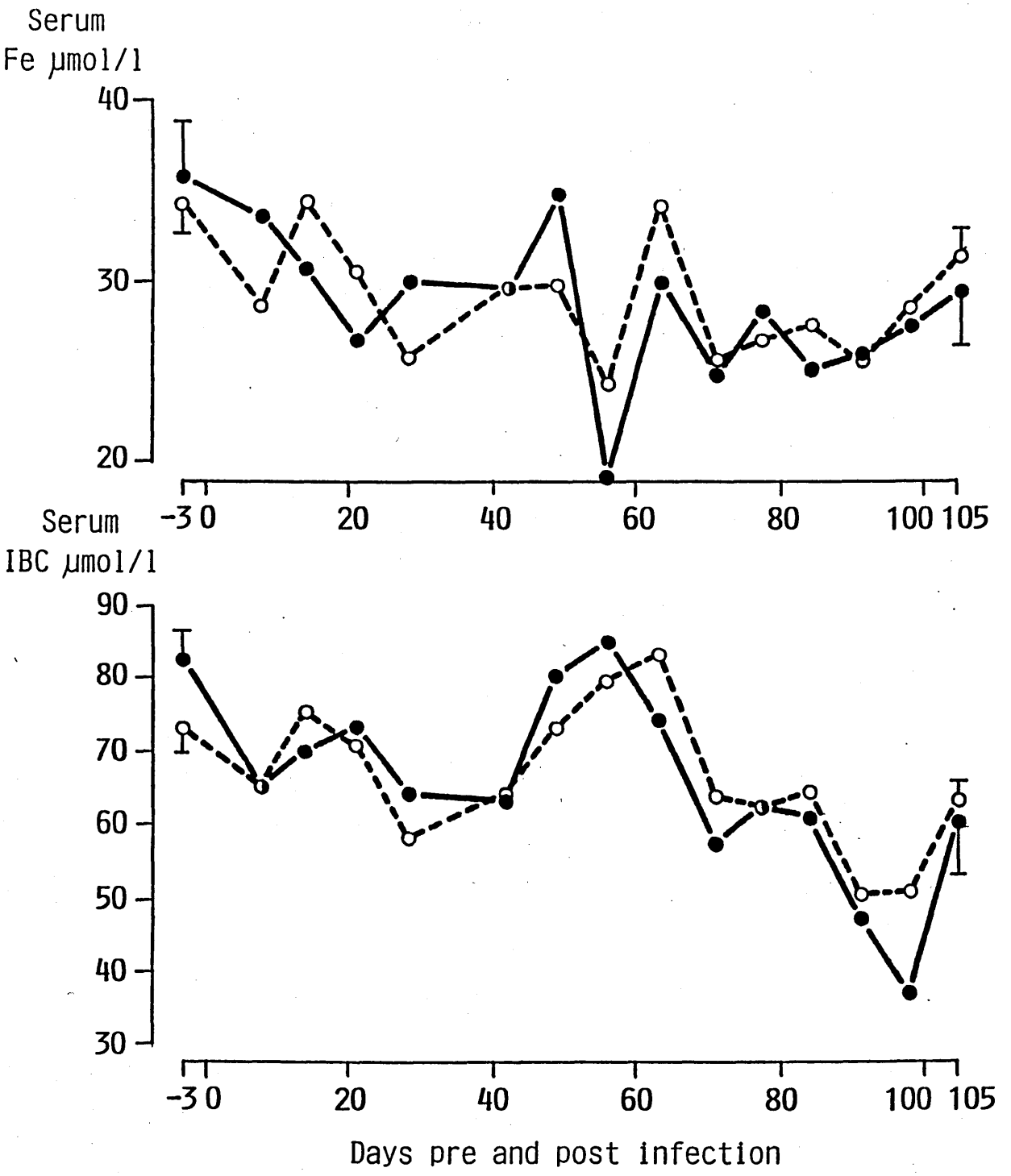

Figure 3b.8 Serum iron (Fe) concentration and iron binding capacity (IBC) of lambs given a trickle infection of H. contortus (●——●) and uninfected controls (O----O).

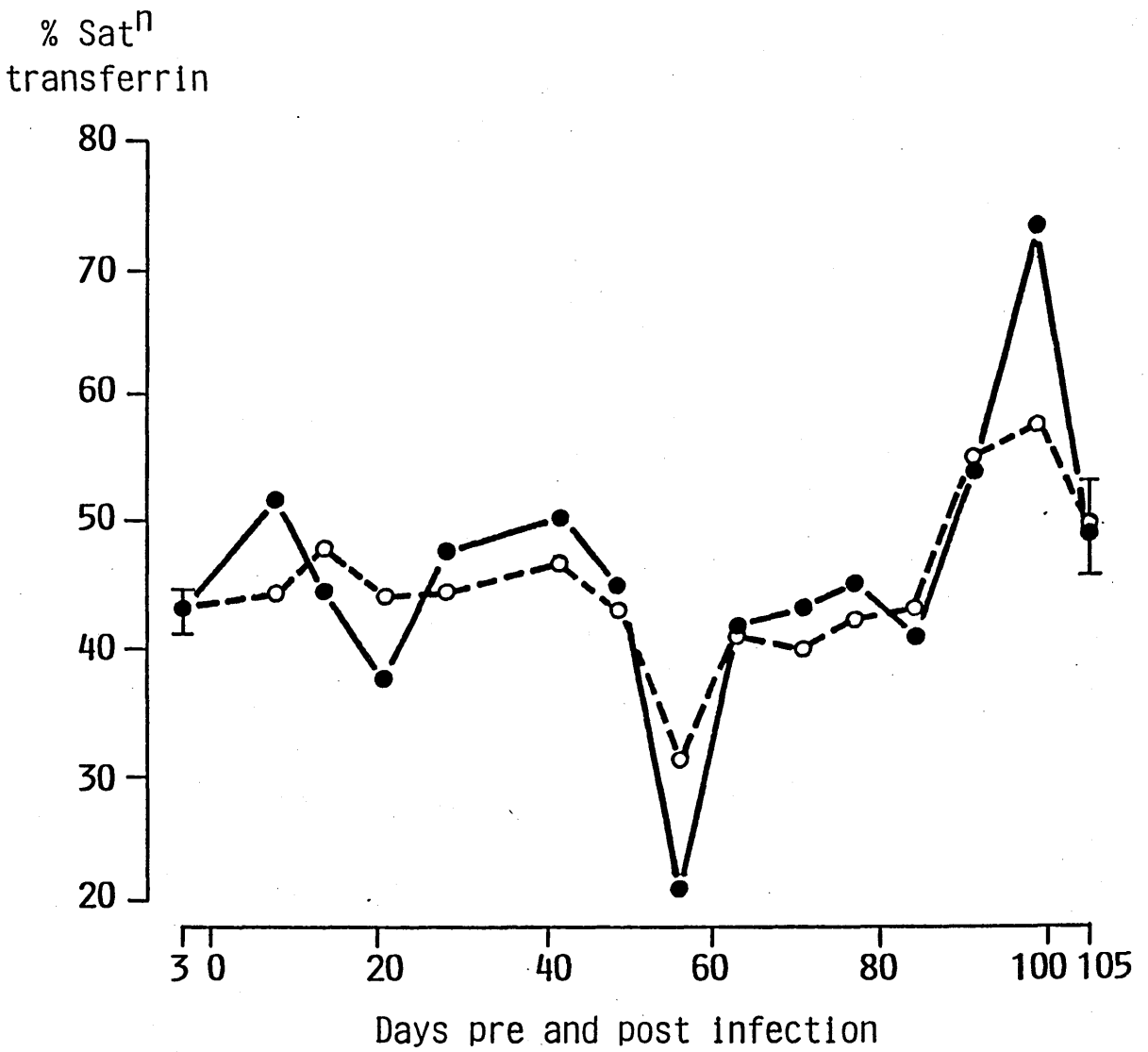

Figure 3b.9 The percentage saturation (% satn) of transferrin of lambs given a trickle infection of H. contortus (●———●) and uninfected controls (O– – –O).

significant (Table 3b.5). The low eye muscle DM coefficient in groups ALI and PFC at 53 DAI were due largely to the very low DMs of the samples from the small lambs ALI-8 and PFC-30 which were 0.195 and 0.211 respectively. The joints of ALC were heavier than those of the other groups at both killings, but again the differences were not significant.

Body composition - chemical analysis

The mean body composition of the groups is shown in Table 3b.6, and the composition of each kg of empty bodyweight gain in Table 3b.7. Large individual variations within each group obscured any infection effect.

Body composition - neutron activation analysis

The amounts of N, Ca, P, Na and Cl per kg empty BWt of eight control sheep were calculated from NAA values of carcase and non-carcase samples, and are presented in Table 3b.8. Equivalent values for N, Ca and P measured by conventional chemical analysis are included to allow comparison between the techniques and it is apparent that some discrepancies exist between the results obtained from the two methods.

Percentage retention of dietary gross energy and crude protein

The percentage retention of dietary GE and CP is shown in Table 3b.9 and there were no significant differences in these parameters between groups or between times of killing. The low values of groups ALI and PFC at 53 DAI reflect the inefficient conversion of feed by the small lambs ALI-8 and PFC-30.

Table 3 b.5

Mean (± S.E.) weight and composition of the right best end neck joint of sheep infected with *H. contortus* (ALI) and of controls pair-fed (PFSC) or offered food *ad libitum* (ALC)

Group	Killed DAI	Wt. of joint (g)	% Bone	% Fat	% Total Lean	% eye muscle	DM eye muscle
ALI	53	361 (64.3)	26.3 (4.88)	25.6 (6.28)	48.1 (2.86)	17.5 (1.62)	0.253 (0.0197)
PFC	53	377 (64.4)	19.9 (1.33)	32.6 (1.89)	47.5 (3.12)	17.8 (0.97)	0.253 (0.0114)
ALC	53	403 (50.4)	24.8 (1.68)	31.1 (3.4)	44.1 (3.11)	17.9 (1.11)	0.328 (0.0426)
ALI	109	505 (42.2)	20.3 (1.59)	34.5 (1.29)	54.3 (2.52)	19.5 (1.97)	NA
PFC	102	503 (37.1)	15.9 (0.44)	39.4 (1.92)	44.7 (1.76)	19.1 (1.03)	NA
ALC	109	524 (52.7)	20.3 (1.22)	35.8 (3.21)	43.8 (3.00)	18.0 (1.59)	NA

DAI Days after infection
NA Not measured

Table 3b.6

Body composition of sheep infected with H. contortus (ALI) and of controls pair-fed (PFC) or offered feed ad libitum (ALC).

Group	ALI	PFC	ALC	ALI	PFC	ALC
Killed (days after infection)	53	53	53	109	102	109
n	5	5	5	5	5	5
Bodyweight (kg)	33.1 (4.38)	34.6 (4.06)	34.2 (2.04)	43.6 (1.24)	44.8 (1.21)	43.7 (3.04)
Empty Bodyweight (kg)	26.0 (3.82)	25.6 (3.16)	27.6 (1.71)	36.8 (0.75)	36.8 (1.11)	35.6 (2.37)
Total body fat (kg)	5.12 (1.082)	5.44 (1.001)	6.20 (0.806)	9.89 (0.526)	10.29 (0.709)	9.86 (1.018)
Body water (% in fat-free empty body)	72.2 (1.15)	73.8 (0.59)	73.3 (0.50)	71.9 (0.34)	71.5 (0.64)	71.0 (0.76)
Body protein (% in fat-free empty body)	19.5 (0.55)	20.3 (0.33)	19.7 (0.24)	21.1 (0.17)	21.7 (0.34)	21.4 (0.30)
Total body protein (kg)	4.07 (0.545)	4.12 (0.494)	4.23 (0.228)	5.68 (0.059)	5.74 (0.170)	5.50 (0.323)

Table 3 b.7

Composition of 1 kg net gain in empty bodyweight of sheep infected with H. contortus for 53 or 109 days (ALI) and of controls pair-fed (PFC) or offered food ad libitum (ALC)

Killed (Day)	53			109*		
Group	Fat (g)	Protein (g)	Energy (MJ)	Fat (g)	Protein (g)	Energy (MJ)
ALI	283 (64)	122 (20.2)	18.5 (2.80)	386 (25.8)	141 (7.6)	18.5 (0.86)
PFC	398 (69.2)	141 (11.0)	19.0 (1.71)	314 (16.0)	144 (12.3)	19.9 (1.09)
ALC	344 (37.7)	126 (4.7)	17.3 (1.45)	355 (25.9)	143 (6.1)	20.0 (1.45)

* Group PFC killed 102 DAI (days after infection)

Table 3b.8

A comparison of neutron activation analysis (NAA) and chemical analysis (chem) of the body composition of eight control sheep.

Sheep No.	N g/kg empty BWt		Ca g/kg empty BWt		P g/kg empty Bwt		Na g/kg empty BWt	Cl g/kg empty BWt
	NAA	Chem	NAA	Chem	NAA	Chem	NAA	NAA
PFC-30	29.78	24.63	12.82	16.40	7.48	4.98	1.30	1.26
FFSC-18	28.55	25.27	10.60	14.86	6.45	4.72	1.09	1.01
PFC-24	30.95	24.00	10.16	9.18	6.50	3.64	1.18	1.15
PFC-46	30.07	25.64	13.07	16.72	7.59	6.11	1.21	1.13
PFSC-16	30.18	24.24	11.74	12.49	6.28	4.85	1.12	0.99
ALC-4	29.04	23.97	11.98	15.47	6.99	5.06	1.13	1.10
PFC-9	29.12	24.54	11.50	9.61	6.70	4.42	1.13	1.07
PPSC-23	29.02	24.85	11.03	11.93	6.58	4.75	1.08	1.07
Mean (± S.E.)	29.59 (0.280)	24.64 (0.210)	11.61 (0.359)	13.33 (1.049)	6.82 (0.173)	4.82 (0.243)	1.16 (0.026)	1.10 (0.030)
ARC (1980)	25.47*		11		6		1.3	0.9

* Protein ÷ 6.25

Table 3b.9

The % retention of dietary GE and CP by lambs infected with H. contortus (ALI) and of controls pair-fed (PFC) or offered food ad libitum (ALC)

Days after infection killed	53	53	53	109	102	109
Group	ALI	PFC	ALC	ALI	PFC	ALC
% Retention of Dietary GE	8.5 (1.34)	7.1 (0.69)	10.8 (0.94)	10.3 (0.74)	10.6 (0.62)	12.1 (0.84)
% Retention of Dietary protein	8.1 (2.14)	7.4 (1.39)	10.3 (0.93)	10.6 (1.68)	10.3 (1.51)	11.6 (1.31)

3.4 Discussion

In these experiments, inoculation of 10-week old parasite-naive lambs with 350 *H. contortus* larvae/kg BWt resulted in patent infections and the lambs became moderately anaemic. Similar lambs, given a trickle infection, developed a milder anaemia. Infection had no effect on feed intake or utilisation, or on growth or body composition. However, the single infection caused an alteration in the digestibility of certain fractions of the diet, six weeks after infection, and nitrogen retention was reduced by both patterns of infection at that time. In other parameters measured the only significant effects of infection were some small changes in hormone concentrations and an increase in blood glucose concentration.

The infected lambs showed no clinical signs attributable to haemonchosis, and grew as well as, or better than, the uninfected sheep. The small infected lamb AL1-8, showed poor growth prior to the experiment so it is unlikely that its poor performance during the experiment was entirely the result of infection. Ideally such small, untypical, lambs should not be used in experiments. However, a shortage of alternative animals necessitated its inclusion to maintain an adequate number of animals in each group. For the same reason, ALI-38 remained in the experiment despite its traumatic reticulitis, but its inclusion did not materially affect the results.

All the infected sheep excreted strongyle eggs, starting between 17 and 21 DAI. The mean excretion from the sheep of group ALSI was considerably greater than that of group ALI sheep, which nevertheless continued to excrete eggs throughout the experiment, albeit at decreasing levels in the later stages. There was no difference between the mean number of worms recovered from group ALI sheep at 53

and 109 DAI, or in the percentage of immature stages found among the recovered worms. This implies that little resistance to infection with H. contortus developed between 53 and 109 DAI, except perhaps in sheep ALI-33 from which only 70 H. contortus were recovered at slaughter. It was interesting that group ALSI sheep had higher faecal egg counts and greater worm burdens than group ALI sheep, despite the latter animals receiving, overall, more larvae than the former.

One control sheep, PFC-2, became infected late in the experiment. The time of egg excretion, and the number of eggs and worms detected, suggests that this sheep was accidentally given one dose of 1000 H. contortus larvae around the 10th week of the experiment. Since this had no effect on the sheep's PCV, feed intake or bodyweight it was considered not to have a significant effect on the sheep's status as an uninfected control.

That infection caused no appetite suppression in this study is in agreement with the findings of Dargie (1973) and Abbott, Parkins and Holmes (1986a). The former author reported that infected sheep actually ate more food than uninfected controls, and in the present experiment the mean intake of the infected sheep also tended to slightly exceed those of both control groups. Abbott et al (1986a) found that lambs infected with 350 H. contortus larvae per kg and offered a high protein ration (170 g CP/kg DM) maintained appetite, while others given similar infections but lower protein feed (88 g CP/kg DM) became anorexic. The sheep in the present experiment maintained appetite on a diet containing 136 g CP/kg DM.

Voluntary feed intakes by the sheep in this experiment exceeded the ARC (1980) predictions for intake of fine, concentrate diets by

up to 30%. This may have been because the ration was pelleted, in addition to being highly palatable.

Around 31 DAI, the mean nutrient intake among goup ALSI averaged 135 g DCP/day and 17 MJ ME/day, when the mean bodyweight was around 35 kg. From the equations of MAFF, DAFS and DANI (1984), this should theoretically have been sufficient to allow maintenance plus 170 g DLG, whereas over this experiment the mean DLG was around 240 g.

Infection had a significant effect on the digestibility of the ration only among the lambs given the single large infection. The reduction in CP digestibility contributed to the reduced N balance in these sheep, but increased urinary N excretion was also a feature. Among the lambs given the trickle infection, N balance was significantly reduced six weeks after infection, but by 14 weeks there was no difference between the groups. The reduction in N balance in these sheep at the first study resulted from a combination of nonsignificant increases in both faecal and urinary nitrogen. Similar effects were reported by Abbott, Parkins and Holmes (1985b) in lambs given 125 H. contortus larvae/kg BWt, but in a later experiment (Abbott, Parkins and Holmes, 1986b) infection with 350 H. contortus larvae/kg BWt had no effect on digestibility or nitrogen retention.

Most of the infected sheep showed changes in haematological indices indicative of anaemia. In general, the anaemia was more severe in group ALSI than in ALI, although the most anaemic sheep overall was the small sheep, ALI-8. The nature of the anaemia, i.e. macrocytic and normochromic was similar to the anaemia described by Dargie and Allonby (1975), Abbott, Parkins and Holmes (1985a) and

Abbott et al (1986a). It is indicative of sub-acute haemmorhage, with a normal host erythropoeitic response.

There was no evidence that the haemoglobin type of the lambs affected the establishment or pathogenicity of the infection in these experiments, in contrast to previous observations by, for example, Altaif and Dargie (1978), but agreeing with the results of the more recent series of experiments by Abbott (1982).

The sheep in the present experiments did not suffer very severe effects of infection, and in discussing this it is useful to compare the protocol and results of the present Experiment 3a with the corresponding features of the work reported by Abbott et al (1986a & b).

In Experiment 3a described here, and in that of Abbott et al (1986a), lambs of similar age and size were given the same number of larvae (350/kg BWt) of the same laboratory strain of H. contortus. Parasite establishment was very similar in the two experiments, with mean worm burdens of 2,648 $\pm$ 687 and 2,962 $\pm$ 521 recorded in the present experiment and that of Abbott et al (1986a) respectively. However, Abbott et al (1986a) reported severe anaemia and deaths among infected sheep. They recorded mean haematocrits of 0.2 and 0.17 l/l four weeks after infection in lambs given high and low protein diets respectively. In contrast, the lowest group mean haematocrit in the present experiment was 0.256 l/l (at 24 and 38 DAI), and no adverse clinical signs of disease were detected.

Two essential differences between the experimental designs may have influenced the severity of disease. These are differences in the breed of sheep selected, and in the diets used.

Abbott et al (1986a and b) used Finn Dorset lambs which

obviously proved susceptible to haemonchosis. In contrast, the lambs in the present experiment were a triple-bred cross (Suffolk x (Border Leicester x Scottish Blackface)). They were thus one-quarter Scottish Blackface, a breed shown to be relatively resistant to the pathogenic effects of H. contortus infection while allowing 'normal' parasite establishment (Abbott et al, 1985a & b).

The other major factor likely to have minimised the effects of infection on the hosts in the present experiment was their relatively high plane of nutrition, a consequence of a high DM intake of a diet of moderate protein and energy content. The mean daily nutrient intake of sheep of group ALSI at 31 DAI was 17 MJ ME and 135 g DCP. In Abbott's experiment (Abbott, 1982; Abbott et al, 1986a) feed intakes were very much lower, and anorexia was a major feature of the disease in the infected group offered the low protein diet. Consequently, daily intakes among the sheep offered the low protein diet were typically around 5 MJ ME and 22 g DCP. Even in the 'high protein' groups, DM intakes were only around 0.9 kg per day, giving nutrient intakes of around 9 MJ ME and 105 g DCP, far lower than in the present study.

Within Abbott's experiment it was apparent that the sheep offered the low protein ration suffered much more serious consequences of infection than those given the high protein ration. By extrapolation it might therefore have been expected that the considerably higher energy and protein intakes of the present sheep would be associated with particularly mild effects of the parasites on the health of these well-nourished hosts.

It is also interesting to compare the present Experiment 3b with

that of Abbott, Parkins and Holmes (1988). Abbott et al (1988) found that among lambs given a trickle infection with H. contortus (amounting to approximately half the larval challenge used in Experiment 3b), those maintained on the low protein diet suffered severe pathogenic effects, while those given the higher protein ration suffered less severe disease and, furthermore, many of the latter lambs developed resistance to further infection. These workers also found that among lambs fed the low protein diet, trickle (Abbott et al, 1988) and single acute (Abbott et al, 1986a) infections were equally pathogenic. In contrast, among lambs given the high protein diet, the trickle infection produced less severe disease than the single infection. As with their 'high protein' groups, it was found in the present experiments that a trickle infection was associated with milder effects than a single large challenge. In addition, although most of the infected sheep in Experiment 3b maintained considerable worm burdens until slaughter, there was evidence of a tendency to recover from the pathogenic effects of the infection in terms of decreasing faecal egg counts and reduced haematological effects.

The results of the limited endocrinological study conducted here tended to show that, in the absence of anorexia, infection with H. contortus was associated with reductions in concentrations of growth hormone and cortisol, an increased prolactin concentration, and no change in insulin level, despite an increase in blood glucose concentration. These changes were not large. Nevertheless, most differences were statistically significant, and in view of the lack of effects of infection on other functions it is perhaps not surprising that only small alterations were detected in the

biochemical parameters measured.

There are no previous reports of endocrine function in ovine haemonchosis, although a few workers have investigated this aspect of other parasitic infections. As discussed previously, Prichard et al (1974) measured hormone changes in sheep infected with T. colubriformis. They found that corticosteroid levels rose in both infected and pair-fed sheep, with the rise being greater in the infected animals. Insulin was depressed in both groups when feed intake fell.

More recently, Fox, Gerrelli, Pitt, Jacobs, Hart and Simmonds (1987) measured some endocrine effects of a single infection with O. ostertagi in calves. They found no effect on cortisol levels, but relative decreases in both growth hormone and insulin concentrations in infected calves compared to pair-fed controls. In a subsequent study of calves infected daily with O. ostertagi (Fox, Gerrelli, Pitt, Jacobs, Gill and Gale, unpublished presentation to AVT&RW meeting, Scarborough, March 1988) they found a rise in growth hormone/insulin ratio associated with a drop in feed intake in infected and pair-fed controls, compared to uninfected ad libitum control animals. Thus even within one host-parasite system there is conflicting evidence of endocrine changes, and as yet no clear pattern is emerging from the sparse data available to support a major role for these metabolic hormones in the pathogenesis of trichostrongylidosis. This is undoubtedly an area which merits further investigation, particularly with haemonchosis where the confounding factor of anorexia is not a common feature.

The infected sheep developed a relative hyperglycaemia, despite higher insulin levels than controls. References to blood glucose

concentrations in parasitised animals are scant, but Bennett, van Dewark and Jackson (1968) found that infection with H. contortus had no effect on blood glucose levels in pregnant or non pregnant ewes.

Considering insulin and growth hormone concentrations, it can be seen that the infected and control sheep offered feed ad libitum had very similar values while those of the pair-fed controls were quite different. As mentioned before, the levels of feed intake were very similar in all three groups, but there was a difference in feeding pattern between the ad libitum and pair-fed sheep. The former were offered fresh feed twice daily in sufficient quantity to ensure they had continual access to food. At feeding times they tended to consume a meal, but they were also observed to eat small amounts at intervals throughout the day and evening (no observations were made at night). The pair-fed sheep, on the other hand, offered feed only once daily, tended to eat most of their daily ration within 20 minutes of its being offered. This behaviour is reflected in the insulin concentrations, which remained fairly high throughout the day in the ad libitum sheep, while among the pair-fed sheep they rose from a low level at 0800 h to a peak at 1000 h after feeding and then gradually fell to their lowest mean concentration by 1800 h.

The high mean concentration of growth hormone in group PFSC can largely be attributed to several very high values for sheep PFSC-18. Growth hormone secretion from the anterior pituitary is episodic and pulsatile, and concentrations may change 10 fold in a few minutes. The stimuli for secretion include the onset of sleep, stress, exercise, fasting or a protein meal (Martin, Mayes, Rodwell and Granner, 1985). It is possible that the high values from PFSC-18,

and occasional high values from other sheep, were the result of a stress reaction to sampling. However, the sheep were accustomed to being handled and bled, and did not appear unduly upset by the procedure. Additionally, these high values did not coincide with high concentrations of cortisol, another hormone whose release is associated with stress. Insulin and growth hormone have antagonistic effects with respect to plasma glucose concentrations, but neither the high insulin levels of the groups ALSI and ALC, nor the low growth hormone concentrations within these groups, explain the higher blood glucose concentrations recorded in the infected sheep.

Prolactin, apart from its role in the initiation and maintenance of lactation, is considered to have growth hormone-like metabolic effects (Martin et al, 1985). However, in this experiment, the infected sheep had the lowest growth hormone values, and also the highest prolactin levels, a situation difficult to reconcile with a potential role for those hormones in the pathogenesis of, or host response to, haemonchosis.

De Silva, Kiehm, Kaltenbach and Dunn (1986) measured serum cortisol and prolactin at 10 minute intervals in sheep accustomed to metabolic cages and sampled either conventionally through an indwelling 10 cm jugular catheter, or remotely via a 4 m long jugular catheter. Both hormones were secreted episodically, and it was found that even after previous adaptation, handling sheep during blood sampling caused a significant elevation in serum cortisol and prolactin levels. These findings perhaps demonstrate the limitations of the present study. The sampling interval was 60 to 120 minutes and thus would not adequately detect rapid changes and

peak concentrations of these hormones. A second criticism might be that the samples were obtained by venepuncture, which it may be considered would be even more stressful to the sheep than the restraint required for sampling via an indwelling catheter. The prolactin levels recorded in the present study (mean 94.6 ng/ml) were considerably greater than those recorded by de Silva et al (1986), whereas the cortisol concentrations (mean 12.06) were only slightly above those obtained by de Silva et al by remote sampling. Whilst differences in the type and age of sheep and in the assay protocols precludes direct comparison between the present study and that of de Silva et al, the low cortisol levels recorded here do tend to suggest that the sheep in the present study were not unduly stressed by the sampling techniques used.

A study by Professor E.J.H. Ford (personal communication), measured cortisol concentrations in blood samples collected via indwelling jugular catheters in trained sheep. In order to assess the effects of venepuncture and other procedures on cortisol concentrations, samples were collected via the catheter before, simultaneous with, or after venepuncture of the contralateral jugular vein, and insertion of a hypodermic needle into the gluteal mass. He found that neither of these procedures altered serum cortisol concentrations.

It may be that the apparent discrepancy between the results of Ford and de Silva et al (1986) represent differing management practices and/or degrees of adaptation by the sheep to the procedures involved. If that is so, then the sheep in the present experiment can probably be considered to have been adequately prepared for the results to be meaningful.

The body composition study, although yielding little evidence of an infection effect, does merit some discussion.

The percentage fat and percentage lean in the best end neck joint were shown to be reliable predictors of total body or carcase fat and protein respectively. Unfortunately, there were no differences in joint composition between groups from which to assess the reliability of these parameters in predicting the effect of parasitism on carcase or body composition.

It became apparent from comparison of body composition data derived from chemical analysis and that arising from NAA that there were major discrepancies between the two methods. It is important to determine the reasons for the differences, and the significance of them in respect of the potential usefulness of the two techniques in the type of experiment described here. With this regard it is useful to compare the results with reference to both their precision and their accuracy.

As the samples analysed by both techniques represent eight different sheep, no valid assessment of the precision of the techniques can be determined from these figures. However, some general comparisons can be made. For N determinations, both methods gave low standard errors, and the scatter of the results is fairly similar. This tight grouping suggests that the sheep did not vary much in N content, and the precision of both techniques was high. An alternative, less likely, hypothesis is that both methods underestimated the variation between sheep in N content.

For P and, more markedly, Ca, the greater variation within the results from chemical analysis suggests that either there was considerable variation in the content of Ca and P in the sheep, and

thus the variance from the chemical analysis data reflects population variance, or else population variance was lower and NAA provided more precise results. From observation of individual Ca values for carcase and non-carcase samples it was apparent that there was more scatter between values than seemed reasonable (ie. 8 to 38 g/kg). In addition, duplicate samples tended to give widely different results. Previous workers have have shown that the coefficient of variation for NAA is less than 4 percent (Sharafi et al, 1983), and it was concluded that NAA probably reflected more precisely population variation in Ca and P content, than did the chemical analysis.

The unreliability of the chemical analyses of Ca and P was investigated. Two problems were identified. It was shown that several batches of samples had consistently high Ca results, and this was traced to a faulty diluter used in the preparation of solutions for analysis. This fault was corrected for future experiments.

The second source of error, that of selecting a representative subsample, proved harder to correct. The finely chopped dried mince, on which the chemical analyses were performed, was found to contain spicules of bone. These were not of sufficient size to affect N or EE assays, but were large enough to distort Ca, P and, to a lesser extent, ash results. In the 1 to 2 g sample used, the inclusion or omission of an extra spicule could affect the Ca and P results by around one-third. To reduce the error, 10 g samples were ether extracted, then ashed, ground twice, and used for Ca and P determination. This tended to improve the repeatability of the results, but in order to obtain satisfactory precision it was considered that multiple assays (eg. four) of each sample would be

required. This proved impractical for this experiment, so attempts to assess Ca and P retentions by the sheep were abandoned, and the Ca and P content of eight control sheep are presented here merely to illustrate the problem, and for comparison with NAA results.

The accuracy of the techniques were also compared. As can be seen, the mean results of NAA and chemical analysis were quite different for all three elements which were compared. The chemical analyses were all done by standard, approved methods (see Section 2.2), but given the sampling problems mentioned above, it was considered that only the Kjeldahl N method was likely to be accurate. It is, of course, inherently difficult to compare the accuracy of two techniques without recourse to a third, standard, procedure. It was decided to compare the present results with the body composition figures of ARC (1980), a standard text on ruminant nutrient requirements. The figures of ARC (1980) are, of course, derived from published data arising from conventional chemical analytical techniques.

In the case of N, the mean value obtained in the present study by chemical analysis is only 3 percent lower than the ARC (1980) figure for male and castrate sheep of equivalent bodyweight. This tends to confirm the accuracy of the Kjeldahl results in this experiment. In contrast, NAA gave a mean N value 16 percent higher than the ARC (1980) value, implying perhaps an overestimation by this technique. In a previous study of total body nitrogen in rats measured by NAA and Kjeldahl digestion, the two methods did give results in good agreement (Preston, Reeds, East and Holmes, 1985).

For Ca and P, NAA appeared to overestimate by 6 and 14 percent respectively. The mean values for Ca and P by chemical analysis

were 21 percent higher and 20 percent lower respectively than ARC (1980) values, which reflects the problems encountered here with these techniques.

For Na, NAA gave a mean value within the range cited by ARC (1980) from the literature, but 12 percent lower than their selected typical value. No data was available to ARC (1980) for the concentration of Cl in growing sheep, but by extrapolating from one reference to Na and Cl in cattle, they assumed a Na/Cl ratio of 1.4, and calculated a predicted Cl concentration in sheep. The NAA data from this experiment gives a Na/Cl ratio of 1.05, suggesting that ARC (1980) may have underestimated the Cl content of sheep.

Comparing NAA and chemical analysis techniques for measuring body composition highlighted the problems of preparing representative subsamples of whole sheep to provide data with sufficient precision to allow the detection of what may be fairly subtle differences in body composition between parasitised and uninfected animals. The subsampling problem was compounded by the fact that relatively small (1 to 2 g) aliquots were used for the chemical analyses. In comparison, NAA was performed on 2 kg mince samples, where the inclusion or otherwise of a few extra bone spicules would be relatively less important. Sykes and co-workers, in their series of papers on body composition in parasitised animals (eg Sykes and Coop, 1976), determined Ca and P on solutions derived from acid digestion of 10 kg mince (Field and Suttle, 1966; Sykes and Dingwall, 1975). While their technique could thus be potentially of higher precision, it was not used in the present experiments because of economic constraint and a lack of facilities for the safe handling of such large quantities of strong acid.

In experiments of the type reported here, the experimental design includes appropriate control animals, and the experimenter is looking for fairly subtle differences between animals within the experiment. Obviously, a highly precise analytical technique will be better able to detect small differences caused by the experimental variable. It therefore seems that NAA, which does not have the sub-sampling problems associated with the chemical analytical techniques used here, should prove to be a useful technique for measuring body compositional changes in parasitised animals. Additionally, of course, the intended development of suitable reference 'phantoms' will make it possible to conduct serial in vivo NAA measurements of sheep, and hence record body compositional changes within each animal over the course of an infection.

The lack of effect of infection on carcase composition and feed utilisation, in the face of measurable effects on the blood picture, is in sharp contrast to the situation described in trichostrongylosis by Sykes and Coop (1976), where sub-clinical infection with T. colubriformis resulted in severe effects on nitrogen and mineral accretion, despite absence of clinical signs in 7 of 8 sheep.

The present findings also contrast with those of Abbott (1982) and Abbott et al (1988) who found that infection with H. contortus resulted in a reduced percentage of muscle in the best end neck joint. They reported that the difference between infected and control sheep was greater among the animals maintained on a low protein diet, compared to those offered a higher protein ration. In this respect, as in others discussed above, the infected sheep in the present experiment more closely resembled those of the high protein dietary groups of the series of experiments reported by

Abbott and co-workers. It might therefore be hypothesised that some form of nutritional stress is required before these effects of infection are manifested on production.

SECTION 4

The influence of feed intake on the pathophysiological and production effects of a single infection with *Haemonchus contortus*.

Section 4

The influence of feed intake on the pathophysiological and production effects of a single infection with H. contortus.

4.1 Introduction

In the previous experiments it was apparent that while sheep infected with 350 H. contortus larvae/kg BWt developed patent infections, the net effect on host function was restricted to the development of a moderate anaemia, and production parameters were not markedly affected. Parasite establishment was comparable with the worm burdens reported by previous workers using the same strain of H. contortus, but the anaemia was less severe (Abbott et al, 1986a). Production parameters (food intake and utilisation, and body composition) were not affected to a measurable extent. In contrast, effects of infection on these parameters were evident in the experiments of Abbott et al, most markably in the lambs offered the lower protein ration.

There are several possible reasons for the lower pathogenicity observed in Section 3, compared to Abbott's work. The lambs used were of different breeds; Suffolk x Greyface, rather than Finn Dorset, and there may have been a breed-related genetic resistance to the effects of infection. Alternatively, the differences in pathogenicity and production effects may have reflected differences in nutritional status. Certainly, within the series of experiments reported by Abbott and co-workers, a consistent feature of their results was that sheep given a low protein diet, and whose total feed intake tended to be lower, suffered more severe pathogenic and

pathophysiological effects of infection than equivalent sheep given a high protein diet.

The present study (Experiment 4) was conducted to investigate the influence of total nutrient intake on the pathophysiological and production effects of haemonchosis. The experiment was designed to compare the effects of a moderate infection, similar to that used in Experiment 3a, in lambs given one of two different planes of nutrition. The two diets comprised a larger or smaller quantity of a feedstuff of constant nutritional value, the feed selected being Super Star cubes, the complete ruminant pelleted diet used in the previous studies (Section 3). The rations were calculated to supply the animals' requirement of energy and protein for maintenance, and to allow daily liveweight gain (DLG) of 100 or 200 g. These levels were selected to complement the previous experiments, where similar lambs offered the same diet ad libitum achieved growth rates of over 200 g/day, and to cover the planes of nutrition experienced by young growing lambs which, although not growing at maximal rate, would not be considered to be under nutritional stress or severe restriction.

The experiment utilised four groups of lambs, comprising infected and pair-fed control lambs at each of the two dietary levels. Measurements of feed consumption and carcase analysis of these, and additional initial control sheep killed at the start of the infection period, allowed examination of dietary and infection factors in a 2 X 2 design.

In addition to measurements of feed utilisation and carcase composition, a radioisotopic study was conducted on certain sheep in order to quantify, and measure the impact of nutrition on, the

pathophysiological changes occuring in the infected sheep relative to the uninfected controls. The application of radioisotopic tracer techniques to the study of red cell and plasma protein metabolism in parasitised animals has been well reviewed by Dargie (1975) and Abbott (1982). These techniques undoubtedly provide an invaluable means of understanding the pathophysiological effects of an infection and, indeed, enable these changes to be quantified to a much more accurate degree than can be achieved by simple extrapolation from haematological indices and conventional parasitological observations.

The three radioisotopes used in this experiment were ^{51}Chromium-labelled erythrocytes, ^{59}Iron-labelled transferrin and ^{125}Iodine-labelled albumin.

Erythrocytes are labelled with ^{51}Cr by incubating whole blood with anionic hexavalent sodium chromate ($Na_2{}^{51}CrO_4$) (Gray and Sterling, 1950) which passes through the red cell membrane and is reduced to cationic trivalent chromium which then binds to the B polypeptide chains of haemoglobin (Pearson, 1963). Labelled cells are returned to the donor by intravenous injection and, by the dilution principle, the red cell volume is calculated. The rate of disappearance of the labelled cells from the circulation, expressed as the apparent half-life (T 1/2), provides information on the red cell lifespan. The term "apparent" is used because a proportion of the ^{51}Cr is lost from the cells by elution prior to their destruction, and the calculated value is therefore an underestimate of the true half-life.

Following normal breakdown of erythrocytes, or following intravascular haemolysis, isotope is not re-utilised and is excreted in the urine. However, if blood is lost into the GI tract, the

isotope is found in the GI contents and, since it is not re-absorbed to any significant extent (Owen, Bollman and Grindlay, 1954), its presence in faeces can be used to quantify the amount of blood loss into the GI tract.

Thus, ^{51}Cr-labelled red cells can give valuable information on the rates and routes of red cell breakdown. For information on erythropoiesis, ^{59}Fe as ferric citrate may be used. Injected intravenously, it labels transferrin (ie. the plasma iron pool) and allows measurement of iron removal from the plasma (T 1/2) and plasma iron turnover (PIT). Most of the circulating iron is transported to the bone marrow for incorporation into haemoglobin of new red cells. The rate of disappearance of labelled iron from plasma, and the speed of its reappearance in circulating red cells, serve as indices of erythropoeitic function. For example, in aplastic anaemia, where erythrocyte production is below normal, PIT is reduced. Where erythropoeisis is accelerated, such as might occur in haemorrhagic anaemias, the disappearance of radio-iron from plasma, and its reappearance in red cells, are more rapid than normal.

Measurement of ^{59}Fe activity in faeces and blood, and blood Hb concentration, allows calculation of the daily loss of Hb-Fe in the faeces. In the absence of any reabsorption of Hb-Fe in the intestine, this figure would be equivalent to Hb-Fe loss into the GI tract. Subtracting the faecal Hb-Fe loss (^{59}Fe data) from the equivalent value obtained from ^{51}Cr data (with which reabsorption is known not to occur) provides an estimate of the extent to which Hb-Fe is reabsorbed in the GI tract (Roche et al, 1957).

Radio-iodine (^{125}I or ^{131}I) has proved a useful label for plasma proteins, and has been widely used to study albumin metabolism

in parasitic infections. An advantage of this label is that the iodine, which is bound to tyrosine, is not reutilised after degradation of the labelled protein, but is quantitatively excreted if uptake of iodine by the thyroid has been blocked by previous administration of inactive iodide.

Following intravenous injection of labelled albumin, and analysis of plasma, faeces and urine for radioactivity, a variety of information about albumin metabolism may be obtained. The plasma volume (PV), or more accurately the albumin space, is calculated using the dilution principle. In addition, the size of the intravascular (CA) and extravascular (EA) albumin pools, and the fractional turnover rate (k) of the albumin within the intravascular pool can be calculated. It is also possible to assess protein loss into the GI tract, but since the label is substantially reabsorbed, faecal radioactivity measurements give a gross underestimation of the true loss. These techniques for determining protein distribution and turnover assume 'steady-state' conditions, ie. that synthesis and catabolism are equal. Since this assumption is not valid in many parasitised animals, the results should be viewed as indices of metabolism, rather than absolute values.

Using the above-mentioned techniques, the following experiment was conducted to investigate in detail the influence of feed intake on the pathophysiological and production effects of a single infection with H. contortus.

Experiment 4 The effects of a single moderate infection with *H. contortus* on lambs kept on a high or low plane of nutrition.

4.2 Materials and methods

4.2.1 Experimental design

Thirty-six Suffolk x Greyface male castrate lambs aged 8-10 weeks were paired on the basis of Hb type and bodyweight and one of each pair was allocated to a high (HF) or low (LF) feeding group. Three weeks later, when the lambs were around 12 weeks of age, each feeding group was further subdivided into three groups of equal mean bodyweight. Four lambs from each feeding group were killed as initial controls (groups HFIC and LFIC; together termed IC). Seven lambs from each feeding group were infected with 350 *H. contortus* larvae/kg BWt (HFI and LFI), and the remaining lambs were maintained as uninfected controls (HFC and LFC), pair-fed to individual infected sheep. The Hb types of the lambs used are shown in Table 4.1. The lambs were maintained in individual pens as described previously. Lambs of the HF group were offered sufficient of the experimental ration to sustain maintenance plus 200 g DLG whilst those of the LF group were offered an amount of the same diet designed to allow maintenance plus 100 g DLG.

The lambs were carefully inspected every day for signs of ill-health. Bodyweights, faecal egg counts, haematological and serum protein changes were measured weekly throughout the experiment.

Three lambs from each group were used for radioisotopic studies from 25 DAI until slaughter at 38 DAI. This involved measurement of circulating red cell, plasma and blood volumes, erythrokinetics,

Table 4.1

The haemoglobin (Hb) types of lambs infected with H. contortus and given a high (HFI) or low (LFI) plane of nutrition and of uninfected controls killed at the beginning of the experiment (IC) and pair-fed controls (HFC, LFC)

Group		Sheep No.	Hb. type	Group		Sheep No.	Hb type
IC		IC-75	B	IC		IC-33	AB
		IC-41	B			IC-62	B
		IC-51	AB			IC-47	B
		IC-8	B			IC-24	B
HFI	*	HFI-37	B	LFI	*	LFI-16	AB
	-	HFI-55	AB		-	LFI-10	B
	(-	HFI-3)	B		-	LFI-36	AB
	-	HFI-44	AB		-	LFI-39	B
	-	HFI-61	B		-	LFI-60	AB
	*	HFI-73	B		*	LFI-5	AB
	*	HFI-7	AB		*	LFI-76	B
HFC	*	HFC-15	AB	LFC	*	LFC-42	AB
	(-	HFC-35)	B		-	LFC-12	AB
	-	HFC-56	AB		-	LFC-68	B
	-	HFC-67	AB		-	LFC-63	AB
	-	HFC-26	B		-	LFC-31	B
	*	HFC-30	B		*	LFC-71	B
	*	HFC-20	AB		*	LFC-17	B

* Used in radioisotopic studies

- Nutritional and carcase evaluation studies

( ) Removed from the experiment - urolithiasis

ferrokinetics and albumin catabolism with ^{51}Cr-labelled erythrocytes, ^{125}I-labelled albumin and ^{59}Fe as ferric citrate.

The remaining four lambs in each group underwent a digestibility and N balance study from 33 to 39 DAI and were killed 42 DAI. Carcase evaluation, comprising indicator joint dissection and chemical analysis of carcase and non-carcase homogenates, was conducted on these lambs and on those of the initial control groups. From this data the amount, and composition, of the net gain by infected and control sheep on each nutritional level was calculated. Also calculated were the feed conversion rate, and the percentages of dietary gross energy and crude protein retained in the body over the course of the infection period.

Details of the techniques used are provided in Section 2.

4.2.2 Feeding

The diet used in this experiment was Super Star cubes, the complete ruminant feedstuff described in the previous chapter. The analysis of the batch used is shown in Table 4.2. Sodium chloride (10 g/kg) was added to the rations to help prevent the development of urolithiasis, since the condition had been diagnosed in two lambs from the same rearing group.

The metabolisable energy concentration of the ration (M/D) was calculated as the product of the measured GE (17.6 MJ/kg DM), the mean digestibility coefficient of the previous experiment (0.618) and the metabolisability of DE calculated for the same feedstuff in the experiment described in Section 5 (0.89). These figures gave an M/D of 9.68 MJ/kg DM.

Using the equations and tables of MAFF et al (1984), daily allowances were calculated, to the nearest 100 g, to supply

Table 4.2

The proximate analysis, and energy value, of SuperStar cubes (Hamlyn Milling, Balgarvie Mill, Scone), a complete pelleted ruminant feed.

DM coeff.	g/kg DM						GE MJ/kg DM
	CP	CF	EE	Ash	NFE	OM	
.880	146	142	42	101	569	899	17.6

sufficient energy for maintenance plus 200 g (HF) and 100 g (LF) DLG, based on the mean initial bodyweight (28 kg), and the predicted BWt at fortnightly intervals throughout the study.

The digestible crude protein (DCP) of the ration was calculated as the product of CP (146 g/kg DM) and the mean CP digestibility measured in Section 3 (0.553), vis. 80 g DCP/kg DM. MAFF (1980) confirmed that the predicted intakes of DCP would be sufficient for the predicted DLG, and would in fact be approximately 30 % greater than the calculated requirements.

Thus, individuals of groups HFIC and HFI were initially offered 1.6 kg fresh matter (FM) per day. At 7 DAI this was increased to 1.7 kg/day for group HFI sheep to provide the additional maintenance requirement of their larger bodyweights at that time. However, when the sheep were moved to metabolism stalls for nutritional or radioisotopic studies the amount offered was reduced to 1.6 kg/day in an attempt to minimise feed refusals, since previous experience (Section 3) suggested that intakes would fall at that time. Group HFC sheep were pair-fed to individuals of group HFI on a weekly basis when housed in pens, and daily when the sheep were moved to metabolism stalls.

Sheep of groups LFIC and LFI were offered 1.1 kg FM/day throughout the experiment. Pair-feeding of group LFC was on a weekly basis when in pens, and daily when in metabolism stalls.

4.2.3 Statistical analysis

Parasitological data, being non-parametric, were analysed using a two-sample rank (Mann-Whitney) test. Correlations were examined by least-squares linear regression or with a rank correlation procedure.

Other results were analysed by analysis of variance using one- or two-way classification, with feeding level (F) and infection (I) as main effects, and interaction, as appropriate. Unbalanced data were analysed using GLIM, the analysis consisting of fitting a lattice of simple additive models and testing firstly for interaction and then, if that was not significant, for F and I main effects. Differences between group means were analysed further using a multiple comparison procedure with Bonferroni correction.

4.3 Results

4.3.1 Clinical and bodyweight changes

None of the infected sheep showed any clincial signs of haemonchosis. Three sheep, HFC-15, HFI-3, and HFC-35, became inappetant around days 27, 28, and 32 respectively. All three sheep showed almost complete anorexia for two or three days, with slight dullness, occasional teeth grinding, and frequent passage of small quantities of urine. Appetite was largely regained over the ensuing week, and the sheep made good recoveries. A diagnosis of subacute urolithiasis was confirmed at post mortem examination of HFI-3, when this sheep was found to have a thickened, hyperaemic bladder containing a small amount of gritty material in normal-looking urine. Post-mortems were not performed on the other two sheep, but it was assumed that they too had had subacute urolithiasis.

Sheep HFC-15 remained in the experiment as a control for the radioisotopic study. However, HFI-3 and HFC-35, which had been destined for nutritional and carcase evaluation studies, were removed from the experiment and their respective feeding partners were paired together.

With the exception of the three individuals mentioned above, the sheep grew well throughout the study, although weight gain was reduced when the sheep were housed in metabolism stalls (Figure 4.1). While the sheep were in pens, average DLG was 298 and 257 g in groups HFI and HFC, and 164 and 147 g in groups LFI and LFC, which was about one-third better than was predicted from the data of MAFF *et al* (1984) at the feeding levels achieved (see later). Over the whole experimental period however, DLGs were around 168, 130, 62 and 58 g in the four groups, slightly below the values predicted from MAFF

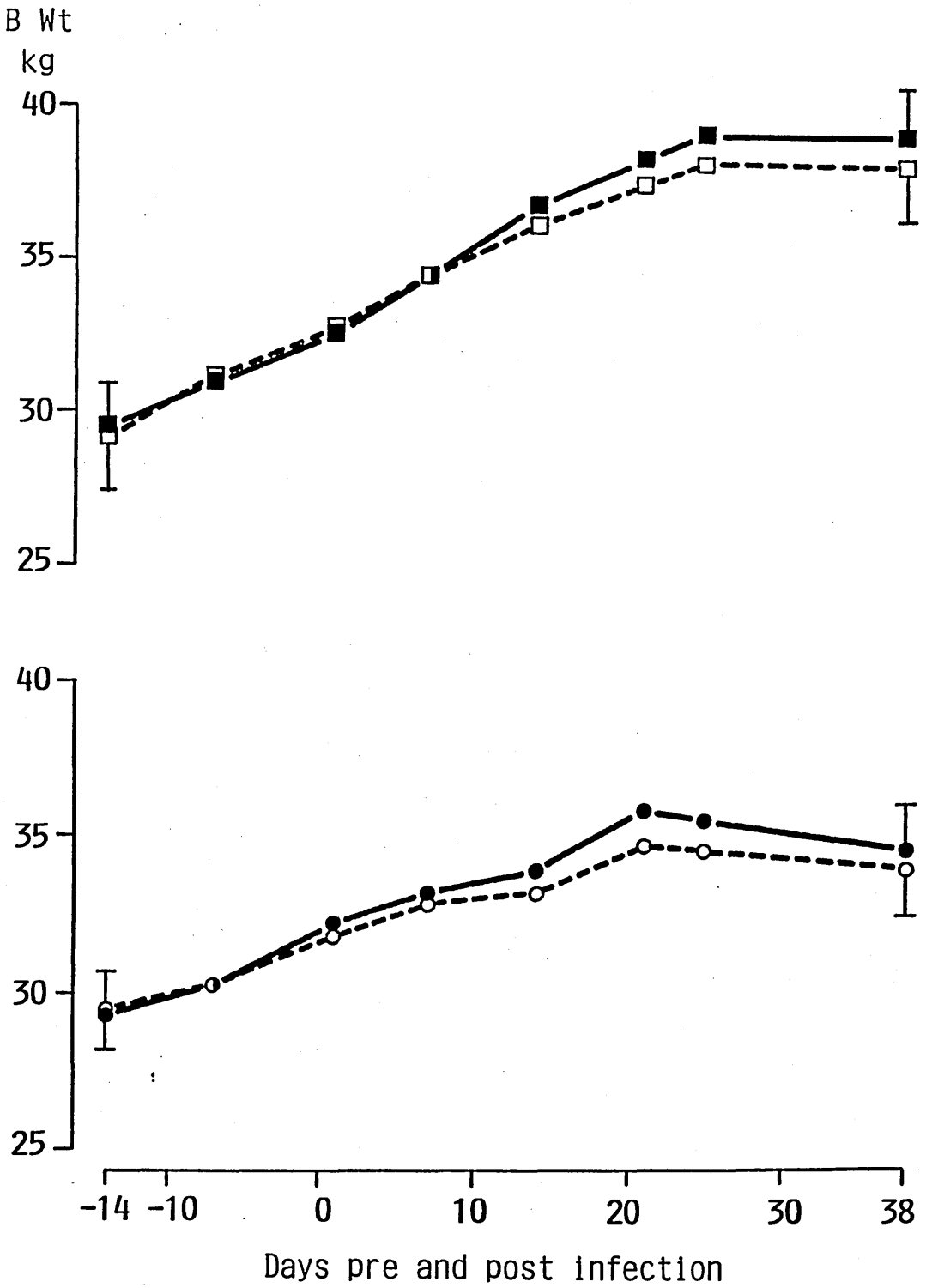

Figure 4.1 Bodyweight (BWt) of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□--□; ○--○).

et al (1984).

4.3.2 Parasitological findings

Infected sheep

Faecal egg counts

Strongyle eggs were detected in the faeces of all the infected sheep by 21 DAI (Figure 4.2). Mean egg counts peaked in both groups at 30 DAI, at 14,430 epg in group HFI and 23,850 in group LFI. Since the daily faecal weights averaged about 1500 and 800 g in the two groups, there was little difference in the mean maximum daily egg excretion between the two groups. The total daily egg outputs for each sheep at 35 and 37 DAI were calculated, and the mean result for each sheep is presented in Table 4.3. The mean total daily output of group HFI at this time was more than double that of group LFI, but because there was so much variation between sheep, the difference was not significant.

Worm burdens (Table 4.3)

All the worms recovered were H. contortus, and all were adults except for 40 and 30 fifth stage larvae from HFI-37 and LFI-60 respectively. The mean number of worms recovered from group HFI was 3243, with a male/female ratio of 1.03, and these worms represented 28.5% of the mean larval challenge. Equivalent values for group LFI were all higher at 3835, 1.24 and 35.0%, but the differences were not significant.

There was no significant correlation between the total egg production at 35 and 37 DAI and the number of worms recovered at 38 or 42 DAI.

Control sheep

Small numbers of strongyle eggs were detected in occasional

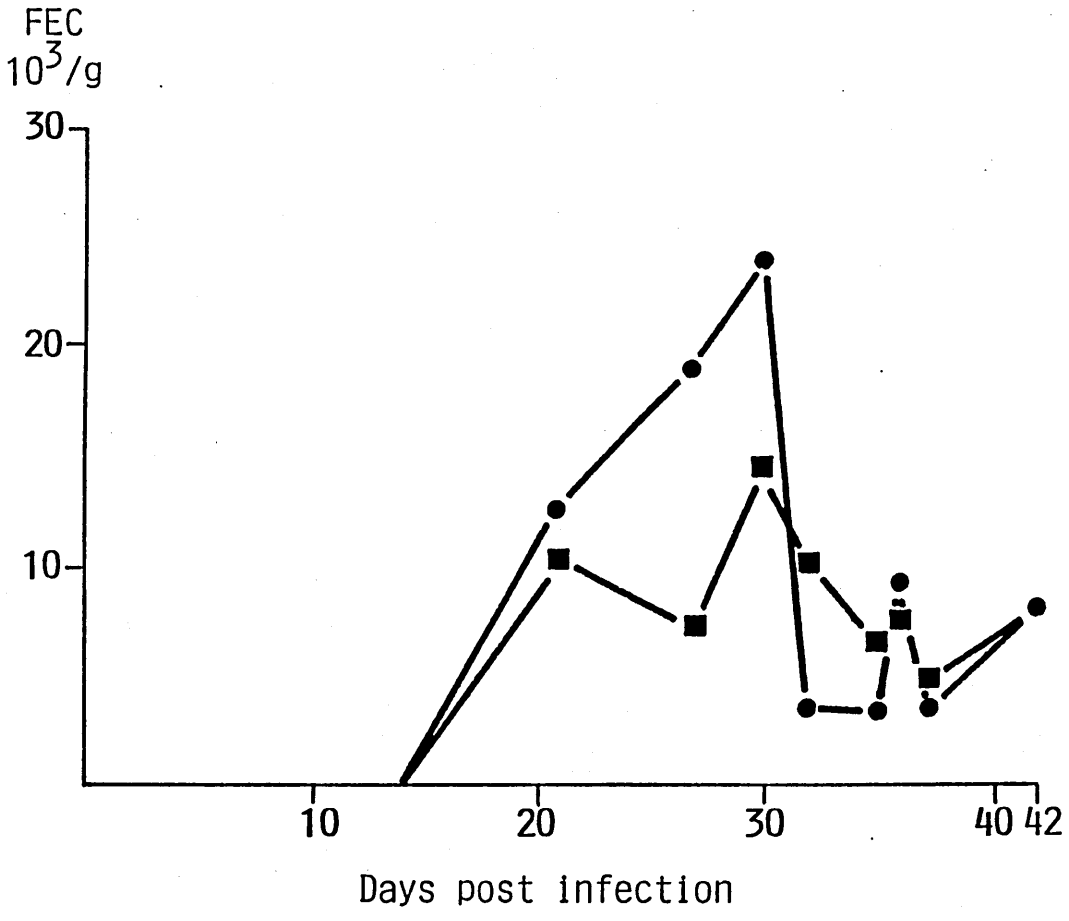

Figure 4.2 Faecal egg count (FEC) of lambs infected with *H. contortus* and given a high (■——■) or low (●——●) plane of nutrition.

Table 4.3

Faecal egg counts and worm burdens of lambs infected with 350 *H. contortus*/kg Bodyweight and given a high (HFI) or low (LFI) plane of nutrition

Sheep No.	Killed DAI	Larval Challenge	Total egg output/day (Mean of 35 and 37 DAI)	Worms recovered at slaughter				Total Worms % of larval challenge
				Larval Stages	Adult Male	Adult Female	Total	
HFI-37	38	13,500	4,639,000	40	2850	2687	5577	41.3
HFI-55	42	12,000	19,021,000	0	2213	2085	4298	35.8
HFI-3	42	11,000	NS	0	1403	1180	2583	23.5
HFI-44	42	11,000	9,225,000	0	1527	1505	3032	27.6
HFI-61	42	10,500	604,000	0	761	381	1142	10.9
HFI-73	38	10,000	9,245,000	0	2102	1620	3722	37.2
HFI-7	38	10,000	12,272,000	0	1196	1152	2348	23.5
HFI $\bar{x}$ (± SE)		11,143 (472)	9,168,000 (2,585,700)	6 (6)	1722 (267)	1516 (278)	3243 (545)	28.5 (3.94)
LFI-16	38	13,000	2,715,000	0	1708	1525	3233	24.9
LFI-10	42	11,500	3,824,000	0	4167	3332	7499	65.2
LFI-36	42	11,000	2,210,000	0	2787	2304	5091	46.3
LFI-39	42	10,500	14,311,000	0	1492	2069	3561	33.9
LFI-60	42	10,500	1,166,000	30	234	239	503	4.8
LFI-5	38	10,000	3,975,000	0	1334	1429	2763	27.6
LFI-76	38	10,000	385,000	0	2106	2090	4196	42.0
LFI $\bar{x}$ (± SE)		10,929 (400)	4,112,000 (1,802,000)	4 (4)	1975 (469)	1855 (358)	3835 (814)	35.0 (7.17)

NS No sample
DAI Days after infection

faecal samples from some control sheep, and a few (<10) adult *H. contortus* were recovered from the abomasa of LFC-63 and LFC-17.

4.3.3 Nutritional studies

Feed intake (Figure 4.3)

Feed intake among group HFI increased steadily from an initial mean value of 1.284 kg fresh matter (FM) per day until by the time of infection, all the sheep except HFI-3 were eating 1.6 kg FM/day. When the ration was increased to 1.7 kg/day, three sheep consumed the complete allowance, while the other four ate at least 1.428 kg. When in metabolism stalls, the ration was cut to 1.6 kg, and the mean intake was 1.534 kg. Intakes by group HFC parallelled those of HFI reasonably well, and the mean daily intakes of the two groups over the infection period were 1.591 and 1.570 in groups HFI and HFC respectively.

These mean values exclude HFI-3 and HFC-35 from the onset of their inappetance, but do include HFC-15 which was transiently anorexic between 27 and 29 DAI, as a result of urolithiasis.

Apart from the first week, and the first few days in the metabolism stalls, when a few lambs of both groups left a little food, each sheep in groups LFI and LFC consumed 1.1 kg/day.

Digestibility study

The digestibility of the proximate fractions of the diet are shown in Table 4.4 and Appendix 2 Table 1. The LF groups tended to have higher digestibility coefficients than the HF groups, the feeding effect being significant with respect to EE and NFE fractions.

Significant interactions between feeding and infection effects were apparent with the other digestibility coefficients. Within

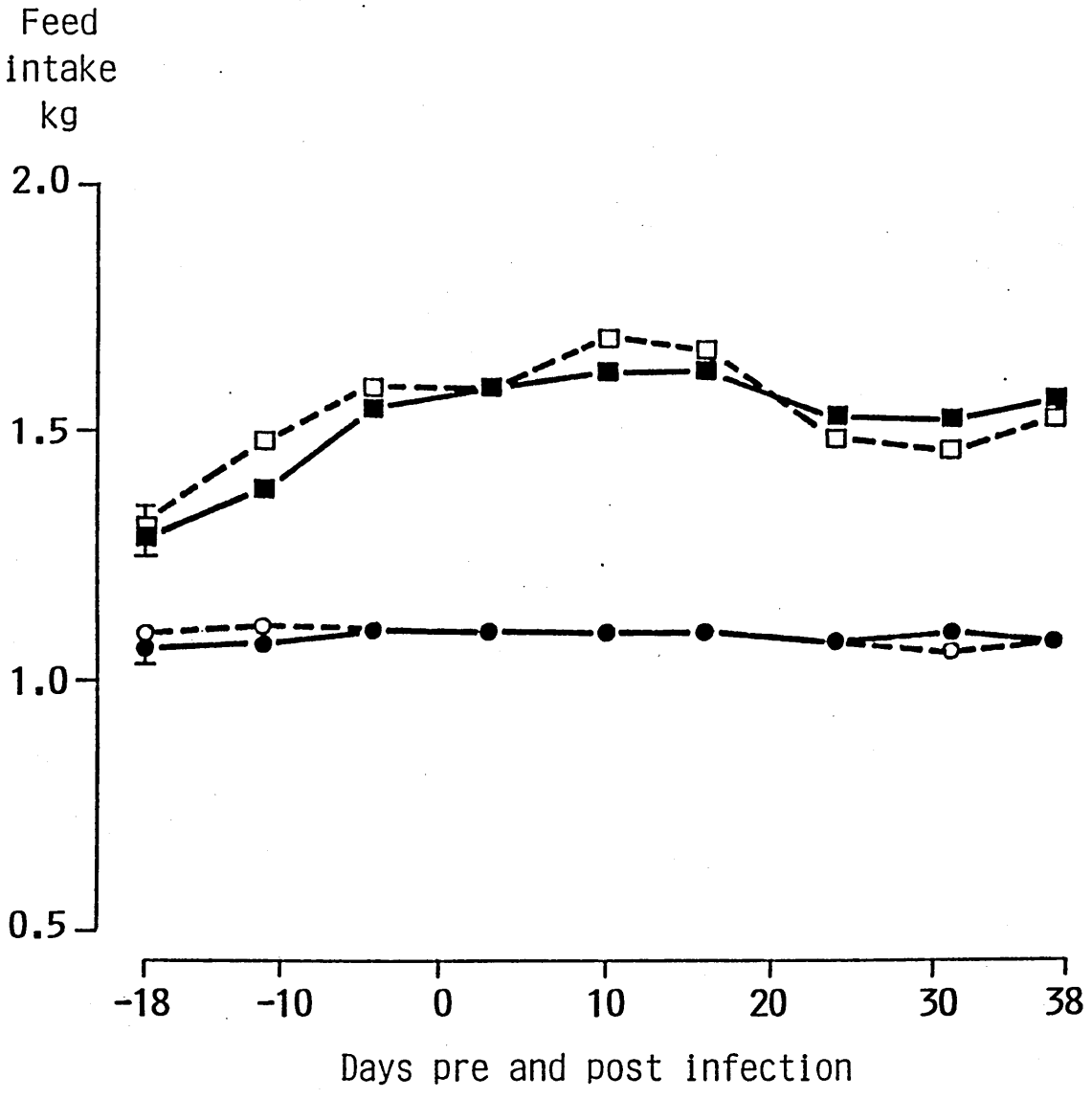

Figure 4.3 Daily feed intake of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□- - -□; ○- - ○).

Table 4.4

Digestibility coefficients of lambs infected with 350 *H. contortus* larvae/kg Bodyweight and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC), measured at 33 to 39 DAI. Figures are mean (± SE).

	n	DM	CP	CF	EE	Ash	NFE	OM	GE
HFI	3	0.579 (0.0087)	0.495 (0.0169)	0.311 (0.0090)	0.752 (0.0164)	0.346 (0.0330)	0.700 (0.0063)	0.606 (0.0077)	0.569 (0.0094)
HFC	3	0.612 (0.0109)	0.531 (0.0229)	0.364 (0.0035)	0.750 (0.0517)	0.413 (0.0069)	0.721 (0.0095)	0.635 (0.0116)	0.601 (0.0173)
LFI	4	0.658 (0.0057)	0.640 (0.0103)	0.426 (0.0041)	0.873 (0.0049)	0.450 (0.0110)	0.742 (0.0081)	0.682 (0.0058)	0.658 (0.0072)
LFC	4	0.641 (0.0061)	0.579 (0.0150)	0.406 (0.0138)	0.846 (0.0108)	0.400 (0.0227)	0.745 (0.0059)	0.700 (0.0048)	0.637 (0.0039)
Significant effects									
Feeding (F)					**		**		
Infection									
F x I Interaction		**	*	**		*		*	*
Groups significantly different at an overall significance level of $P < 0.05$				HFI < HFC					

* $P < 0.05$
** $P < 0.01$

feeding group LF, the infected sheep had higher digestibility coefficients than the controls, whereas within feeding group HF, the infected sheep had lower coefficients of digestion than the controls, and with Crude Fibre this difference was significant.

Nitrogen balance (Table 4.5 and Appendix 2 Table 2)

All sheep had positive N balances, ranging from + 6.1 to + 15.6 g/day.

Within dietary group HF (mean intake 32 g N/d), both faecal and urinary N output were greater in the infected sheep than the controls, resulting in a lower N balance in the former sheep (mean 8.9 v 11.6 g/d), although the difference was not statistically significant.

Within dietary group LF (22 g N/d), urinary N was slightly greater, but faecal N was slightly less in the infected sheep relative to controls, and overall a slightly increased N intake resulted in a marginally higher mean N balance in the infected sheep (8.4 v 7.4 g N/d).

4.3.4 Haematological changes

Both groups of infected sheep developed moderate macrocytic, normochromic anaemias.

Haematocrits of groups HFI and LFI fell sharply between 7 and 18 DAI, from 0.351 to 0.258, and 0.376 to 0.288 l/l respectively (Figure 4.4). Thereafter PCVs continued to fall gradually, reaching minimum values of 0.246 and 0.257 at 35 DAI. Both control groups showed slight falls in PCV from around day 20 until the end of the experiment, but with the mean values remaining above 0.3 l/l. PCVs of both infected groups were significantly lower than their respective controls from 18 to 35 DAI.

Table 4.5

Group mean (± SE) nitrogen (N) intake, faecal and urinary losses and N retention of lambs infected with 350 H.contortus larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC); measured at 33 - 39 days after infection.

Group	n	N intake g/day	N in faeces g/day	N in urine g/day	N retention g/day
HFI	3	31.6 (0.79)	15.9 (0.64)	6.8 (0.70)	8.9 (0.84)
HFC	3	31.8 (0.49)	14.9 (0.72)	5.3 (2.00)	11.6 (2.10)
LFI	4	22.3 (0.22)	8.0 (0.24)	5.9 (0.49)	8.4 (0.71)
LFC	4	21.5 (1.02)	9.0 (0.50)	5.0 (1.00)	7.4 (0.76)
Significant effects					
Feeding (F)		**	*		
Infection (I)					
F x I Interaction					

* $P < 0.05$
** $P < 0.01$

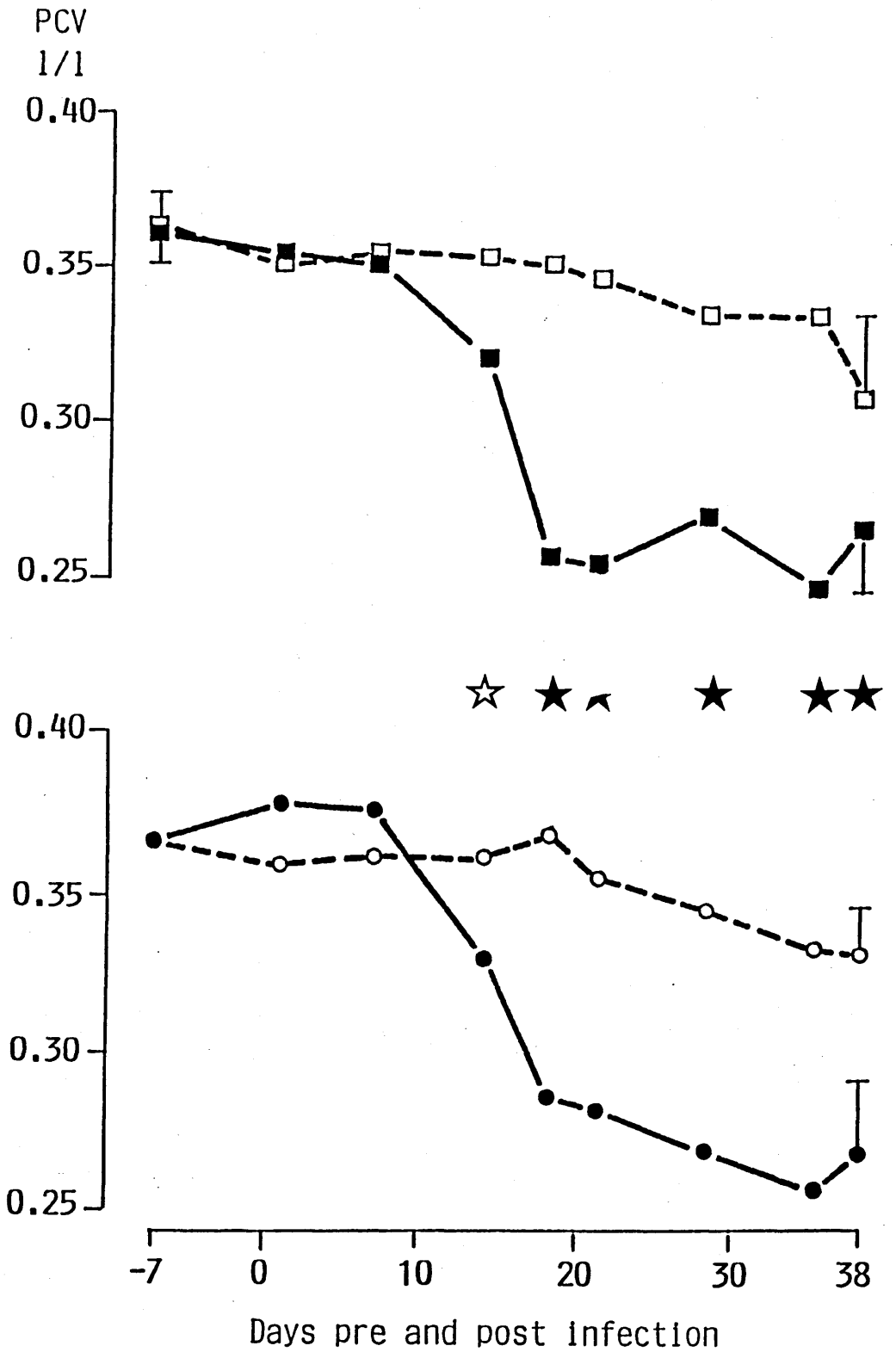

Figure 4.4 Packed cell volume (PCV) of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□---□; ○---○).

Significant effects: Infection ☆ $p<0.05$

" ★ $p<0.01$

There was considerable individual variation in the effect of infection on PCV. In group HFI, the lowest PCV was recorded in HFI-37 (0.179, 38 DAI), while the PCV of HFI-44 was the highest, not falling below 0.28 1/1. In group LFI, LFI-16 developed the lowest PCV (0.2 at 35 DAI) while LFI-60 maintained its PCV at or above 0.328 throughout the infection. In general, the PCVs of the sheep used in the radioisotopic study fell during the study, while over the same period PCVs of the nutritional study sheep were tending to recover toward normal values.

Red cell counts (Figure 4.5) and Hb values (Figure 4.6) tended to parallel the changes in PCV, with marked drops in both infected groups by 14 DAI, and slight drops in the control groups after 22 days. Preinfection RCCs were typically around 12.5 (HF) to 13.5 x 10^{12}/l (LF), falling to 7.3 and 8 x 10^{12}/l in the infected groups at 35 DAI. The effect of infection was significant from 14 to 38 DAI. In the case of Hb, values fell from preinfection levels of 12.8 (HF) and 13.1 g/dl (LF) to 8.2 and 8.8 respectively in the infected groups at 35 DAI, and here again the infection effect was statistically significant from 14 DAI.

The mean MCV of both infected groups remained at preinfection values (below 0.29 fl) until 14 DAI after which the values increased over 15 days to 33.0 and 33.3 in groups HFI and LFI respectively (Figure 4.7). In contrast, MCVs of the control sheep remained around 29 (HFC) and 28 fl (LFC) throughout the experiment. The effect of infection was significant from 21 DAI, and a small but significant difference due to feeding level was observed at 14 DAI.

The MCH values paralleled the changes in MCV (Figure 4.8), with the increase due to infection being significant from 21 DAI. The

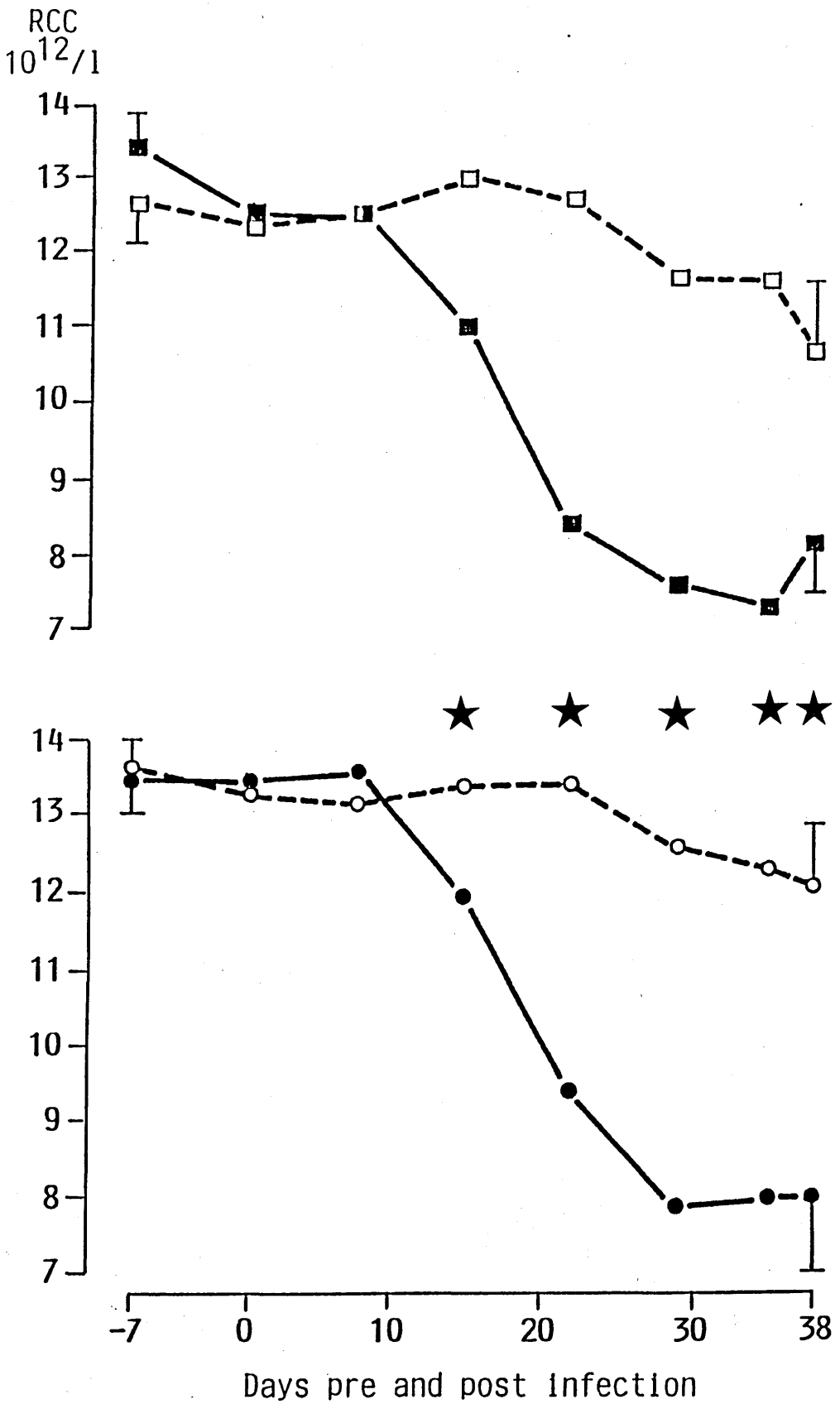

Figure 4.5 Red cell count (RCC) of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□---□ ; O--O).

Significant effects: Infection ★ $p<0.01$

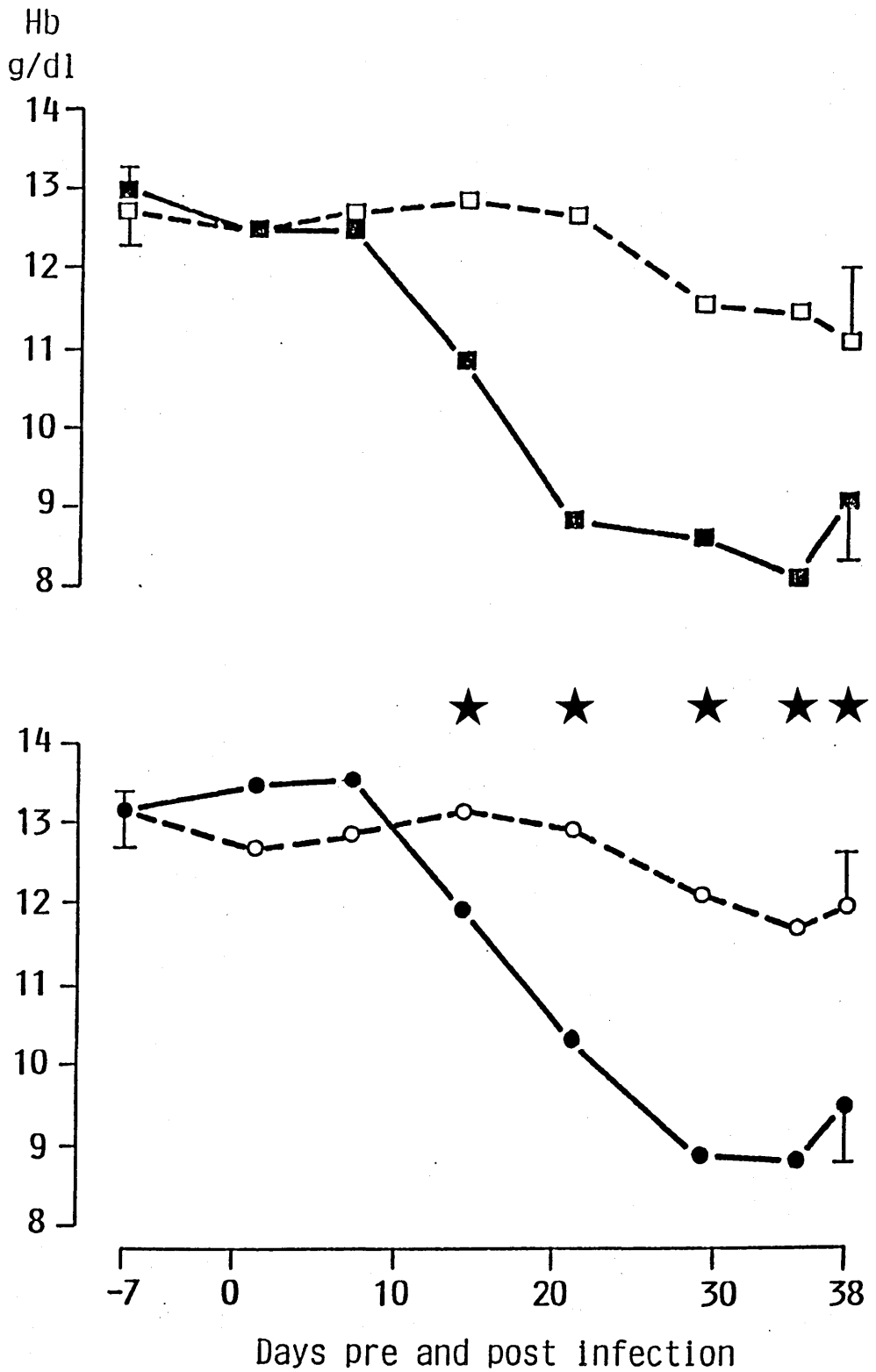

Figure 4.6 Haemoglobin (Hb) concentration of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□---□; ○---○).

Significant effects: Infection ★ $p<0.01$

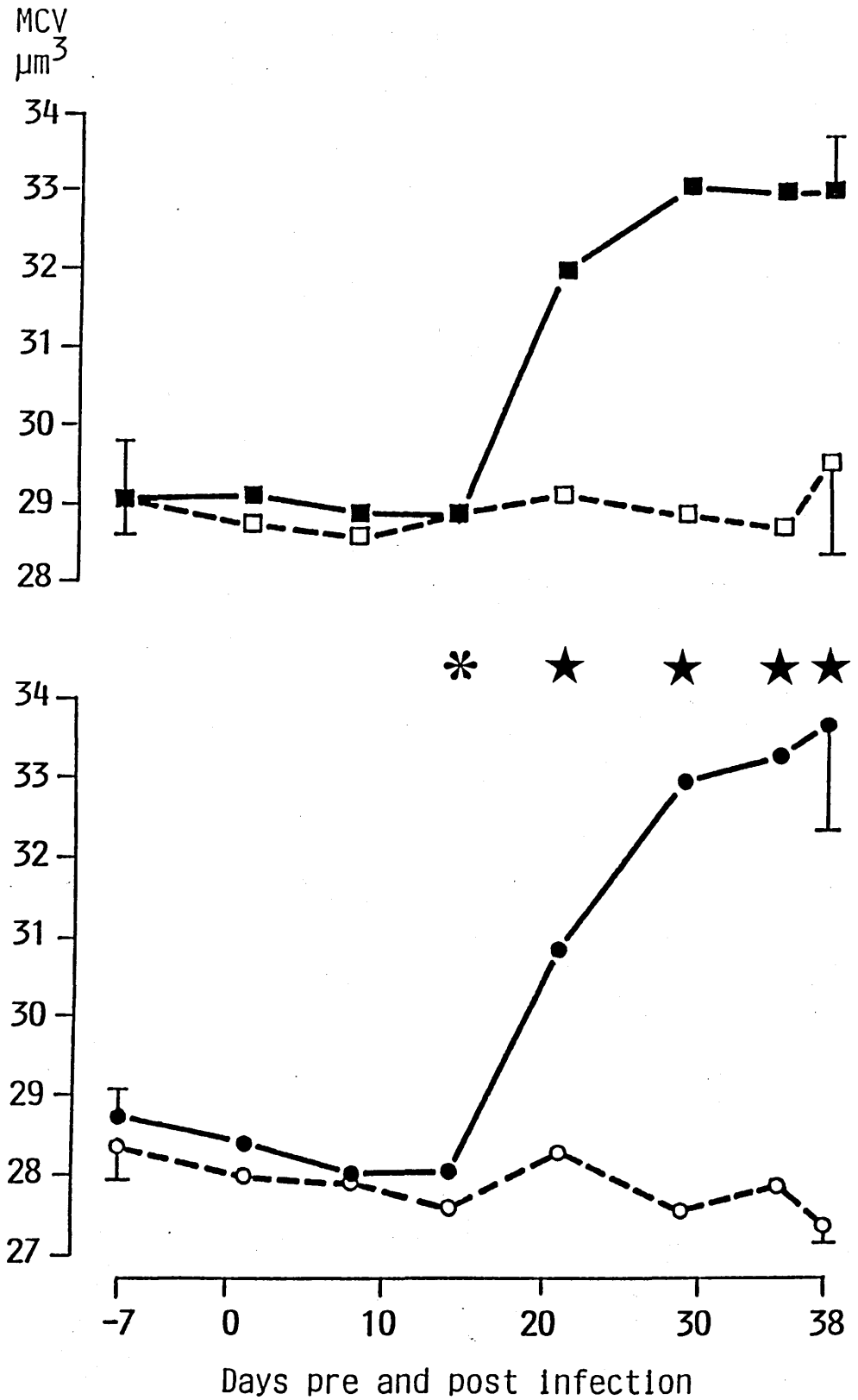

Figure 4.7 Mean cell volume (MCV) of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□– –□; ○– –○).

Significant effects: Infection ★ $p<0.01$

Feeding level ✻ $p<0.05$

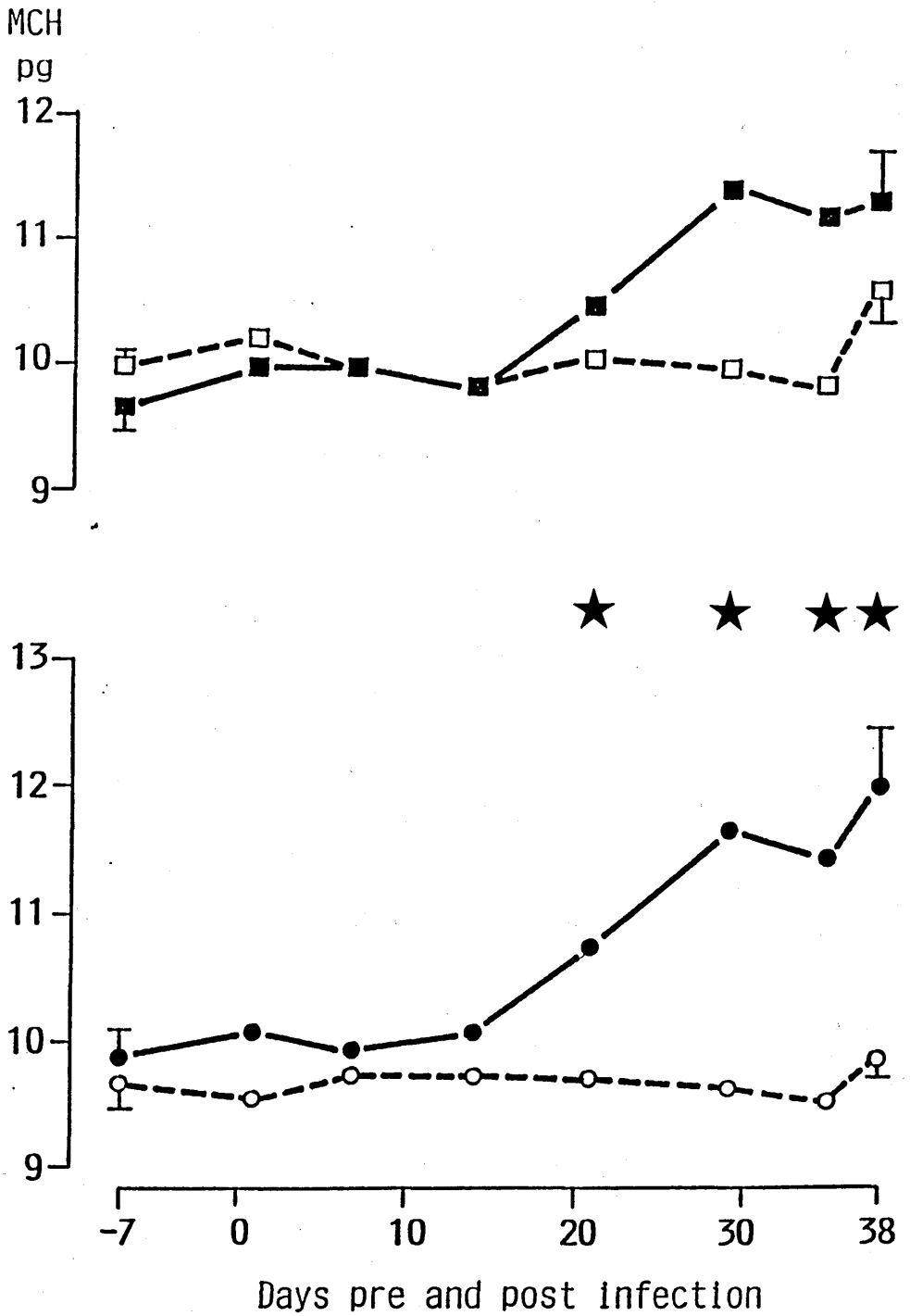

Figure 4.8 Mean cell haemoglobin (MCH) of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□---□; ○--○).

Significant effects: Infection ★ $p<0.01$

MCHC in each group fluctuated a little, but there were no consistent differences between groups (Figure 4.9).

White cell counts fell in all groups over the course of the experiment, with the drop being most marked in the two groups of infected sheep where values of 6.9 and 5.6 x 10^9/l were recorded in groups HFI and LFI, 35 DAI (Figure 4.10), these being significantly lower than those of the uninfected controls.

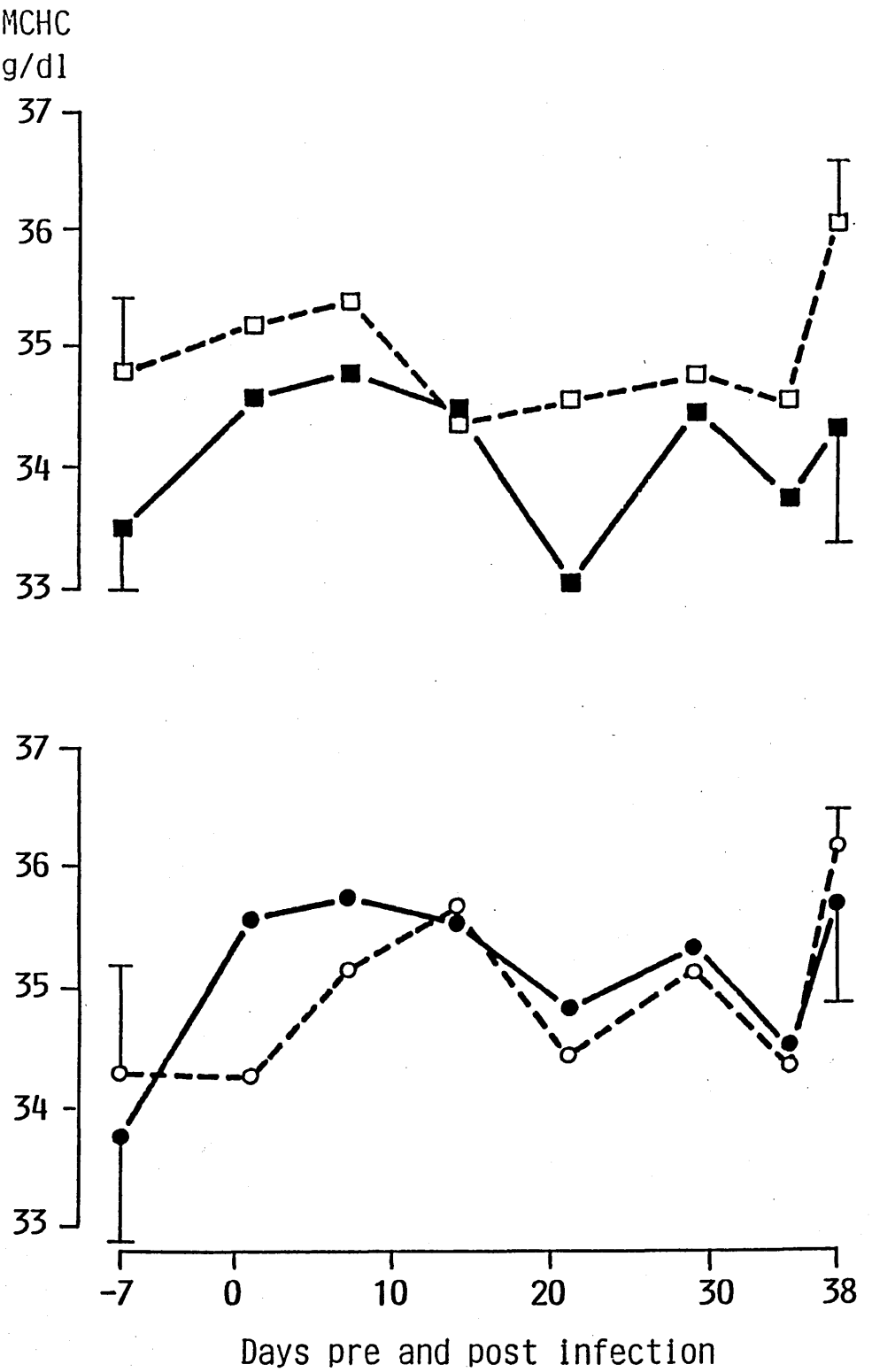

Figure 4.9 Mean cell haemoglobin concentration (MCHC) of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□- - -□; ○- - -○).

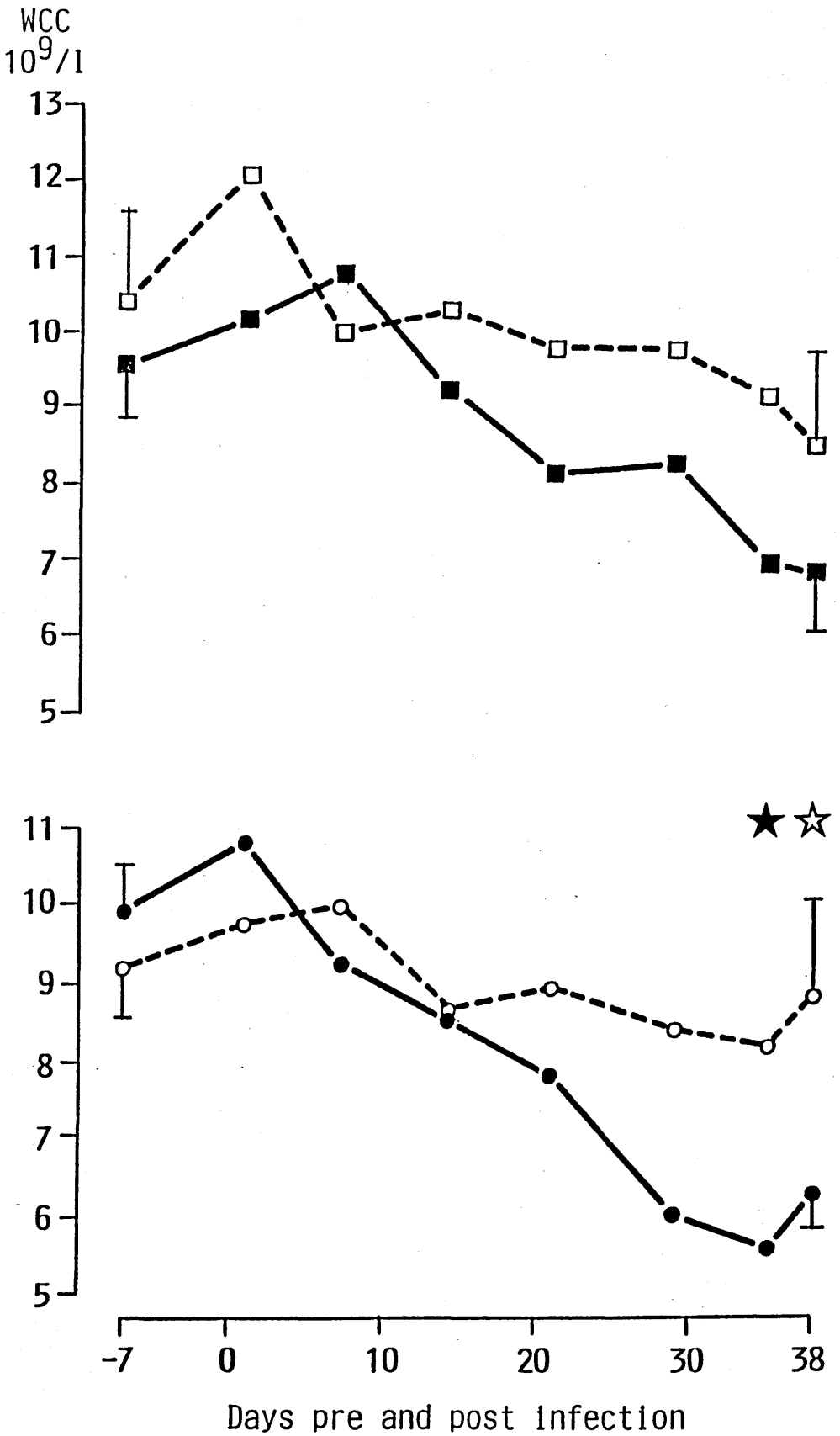

Figure 4.10 Total white cell count (WCC) of lambs infected with *H. contortus* and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□– – –□ ; O– – –O).

Significant effects: Infection ☆ $p<0.05$

" ★ $p<0.01$

4.3.5 Biochemical changes

Serum proteins

Both groups of infected sheep developed a significant hypoproteinaemia by 14 DAI, compared to their controls (Figures 4.11 and 4.12), with total protein values in both infected groups averaging 50 g/l compared to around 60 g/l in the controls. Considering albumin levels, all the sheep showed a slight drop around 7 DAI. In the control sheep, values then returned to previous levels (around 35 g/l), while among the infected sheep the values remained significantly lower at approximately 30 g/l. Globulin values decreased slightly in all the groups, most markedly in the infected sheep where they were significantly lower from 14 DAI.

Serum iron and iron binding capacity

In this study, infection tended to decrease serum iron concentration, IBC and the percentage saturation of transferrin. In addition, there were feeding effects, with the higher feeding level being associated with lower serum Fe and transferrin saturation, and higher IBC.

Serum iron concentrations of group HFI were lower than those of group HFC (Figure 4.13), and toward the end of the experiment the difference was quite marked with values of 23 and 34 umol/l being recorded in groups HFI and HFC at 38 DAI. Among the LF sheep values tended to remain above 30 umol/l with only a small decrease among the infected sheep around 28 DAI. Statistical analysis showed that the effect of infection was significant at 35 and 38 DAI.

Serum IBC tended to be lower, sometimes significantly so, in the infected sheep than the controls. Also, IBC was generally lower among the LF sheep than those on the higher nutritional plane and

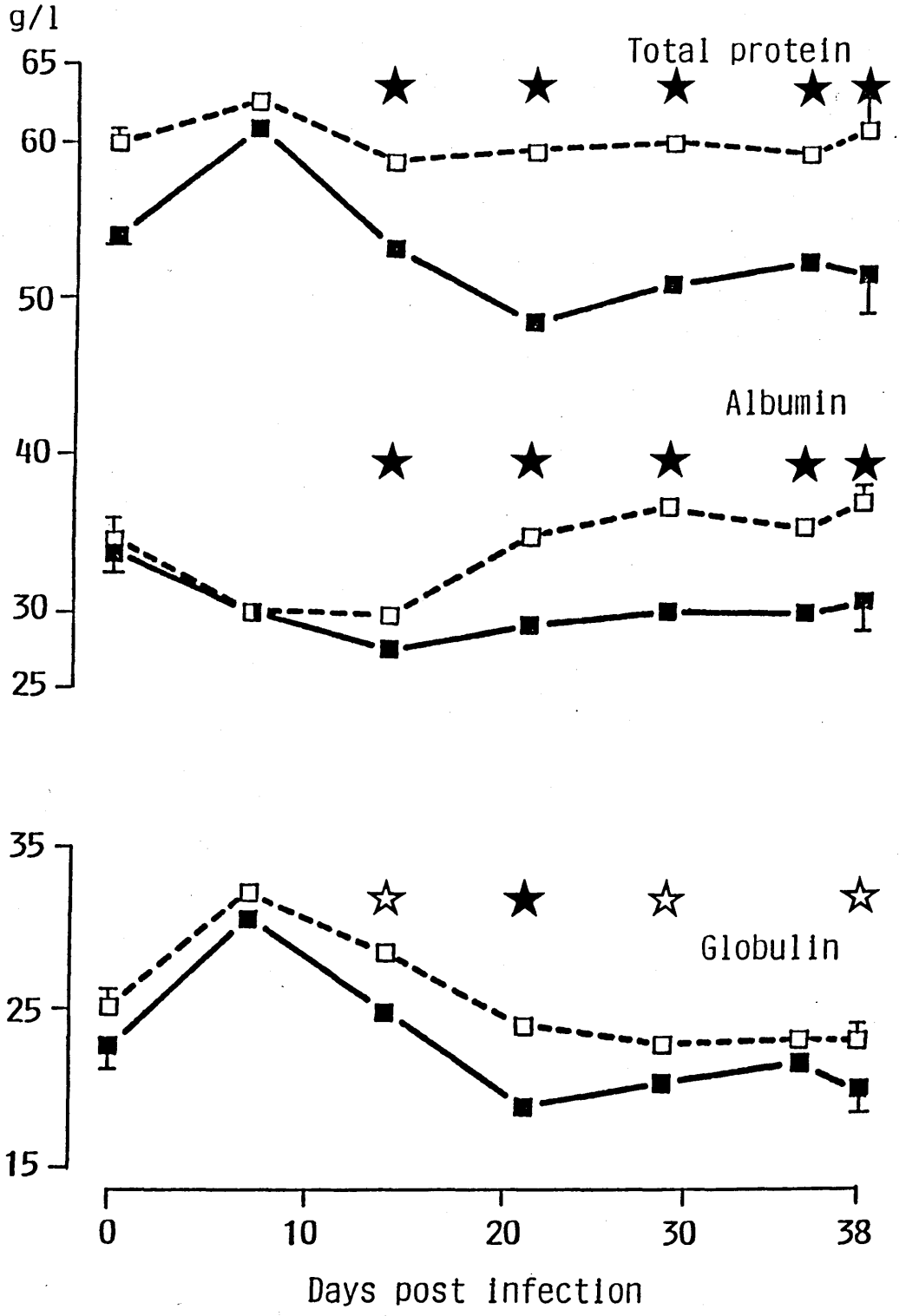

Figure 4.11 Serum total protein, albumin and globulin concentration of lambs infected with H. contortus and given a high plane of nutrition (■————■), and pair-fed controls (□– – –□).

Significant effects (data of Figures 4.11 & 4.12 combined):

Infection ☆ $p<0.05$

" ★ $p<0.01$

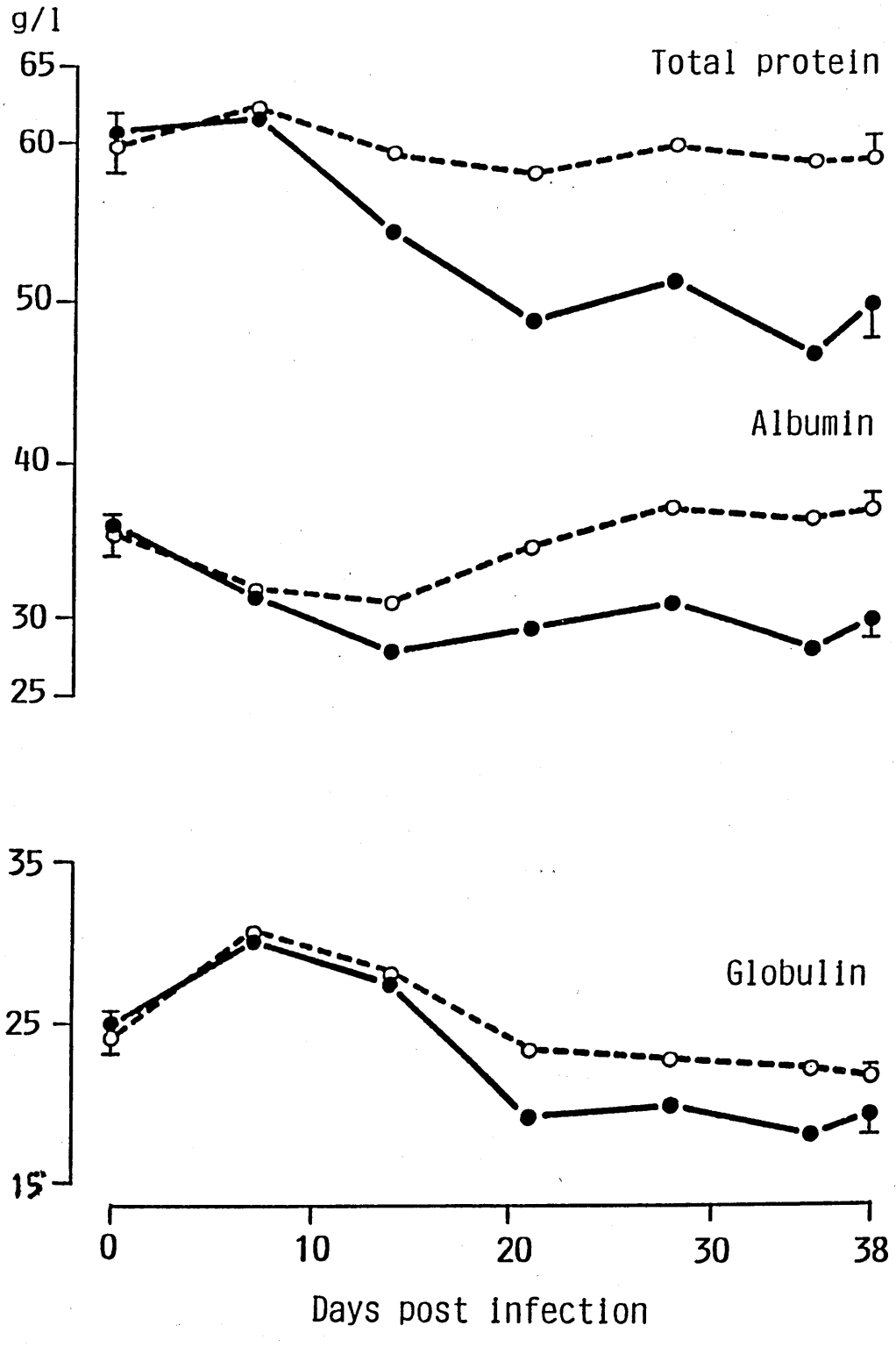

Figure 4.12 Serum total protein, albumin and globulin concentration of lambs infected with H. contortus and given a low plane of nutrition (●——●), and pair-fed controls (O– – –O).
Significant effects are shown on Figure 4.11.

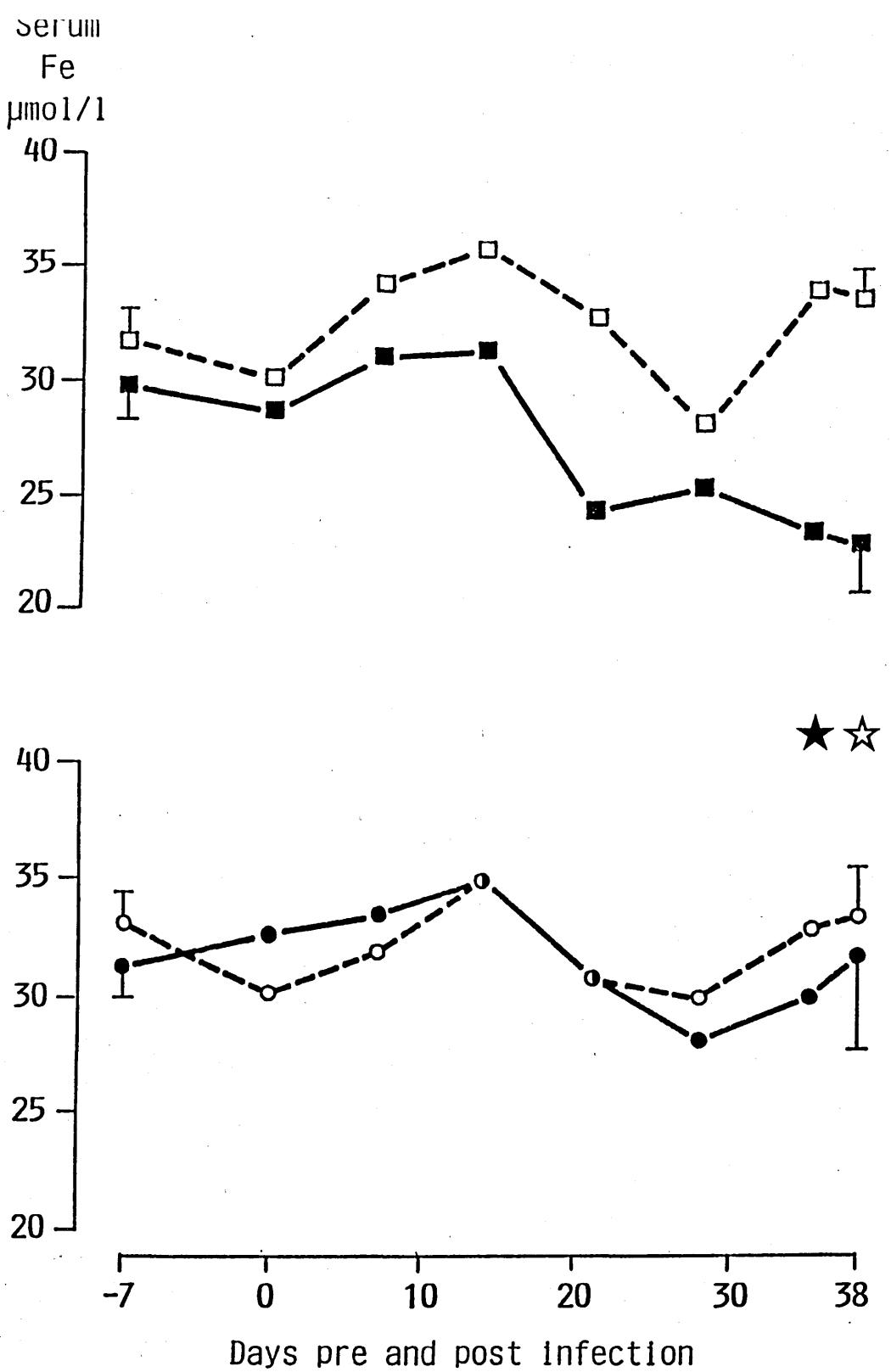

Figure 4.13 Serum iron (Fe) concentration of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□---□; ○---○).

Significant effects: Infection ☆ $p<0.05$

" ★ $p<0.01$

this feeding effect was significant at 35 DAI (Figure 4.14).

The percentage saturation of transferrin with iron was significantly affected toward the end of the study by both feeding level (at 35 and 38 DAI) and infection (at 38 DAI) and because the decrease due to infection was particularly apparent in group HFI, a significant I x F interaction was evident at 38 DAI (Figure 4.15).

4.3.6 Radioisotopic studies

Blood and plasma volumes

The mean circulating red blood cell volume (Table 4.6 and Appendix 2 Table 3) was significantly reduced by infection in both groups of infected sheep (15.5 and 13.5 ml/kg BWt; HFI and LFI) compared to controls (21.9 and 20.7 in HFC and LFC). Infection had less effect on plasma volumes, with values for both groups of infected sheep (49.6 and 46.6 ml/kg BWt in HFI and LFI) being slightly lower than their respective controls (51.2 and 48.5). Whole blood volume was significantly reduced by infection. Comparing the dietary groups, values of these indices tended to be lower within the LF group than the HF group in both the infected and control sheep, although the differences were not significant.

Erythrokinetics

The apparent half-life of circulating red blood cells was significantly reduced by infection in both groups of infected sheep, compared to the controls. A significant F x I interaction was observed, as among the infected sheep group LFI values were lower than those of group HFI, whereas among the controls, the LF sheep had longer half-lives than their HF counterparts.

The mean losses of RBC into the GI tract were respectively 40, 2, 32 and <1 ml/d in groups HFI, HFC, LFI and LFC. Losses were thus

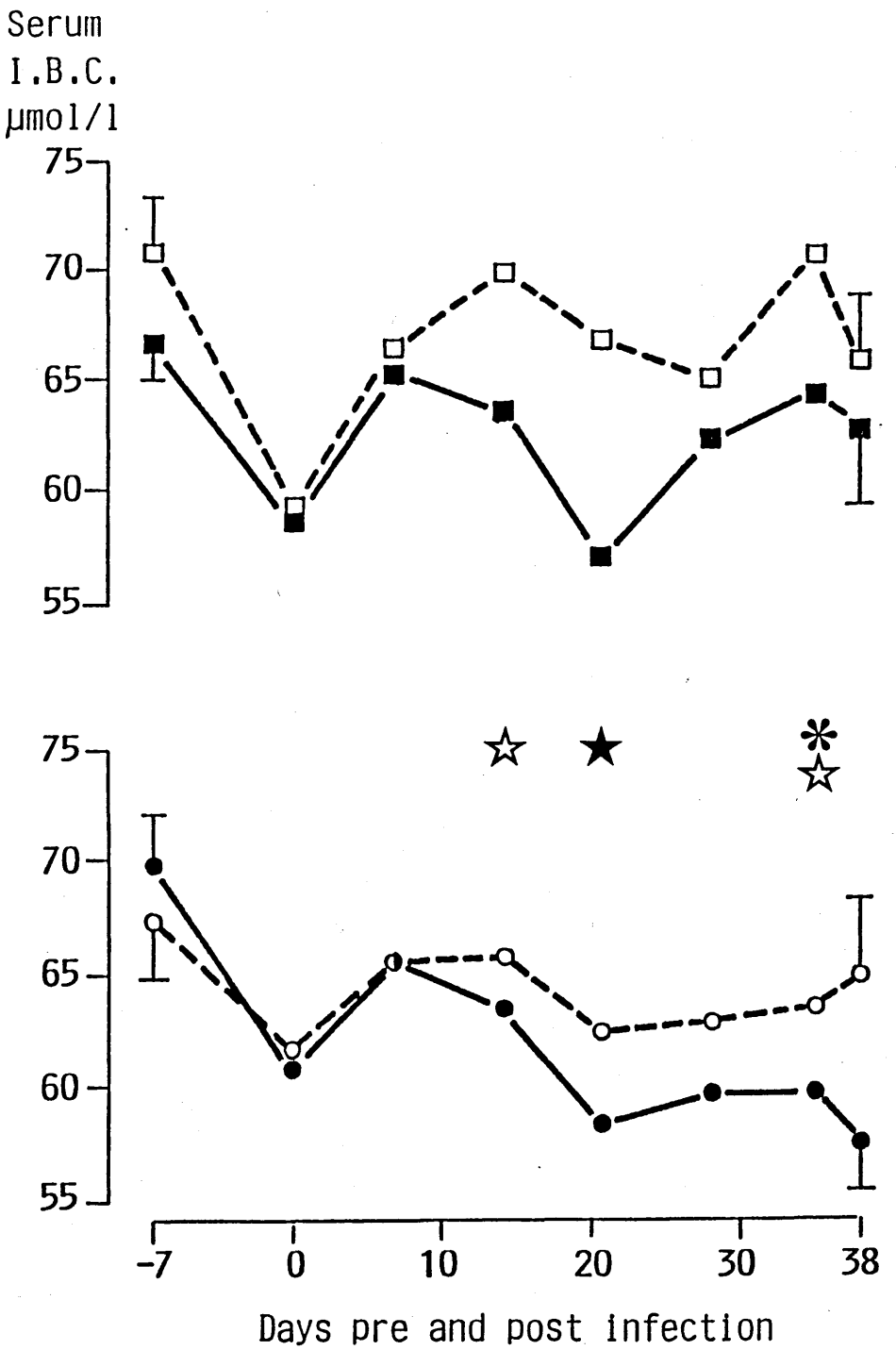

Figure 4.14 Serum iron binding capacity (IBC) of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□---□; ○---○).

Significant effects: Infection ☆ p<0.05
" ★ p<0.01
Feeding level ✻ p<0.01

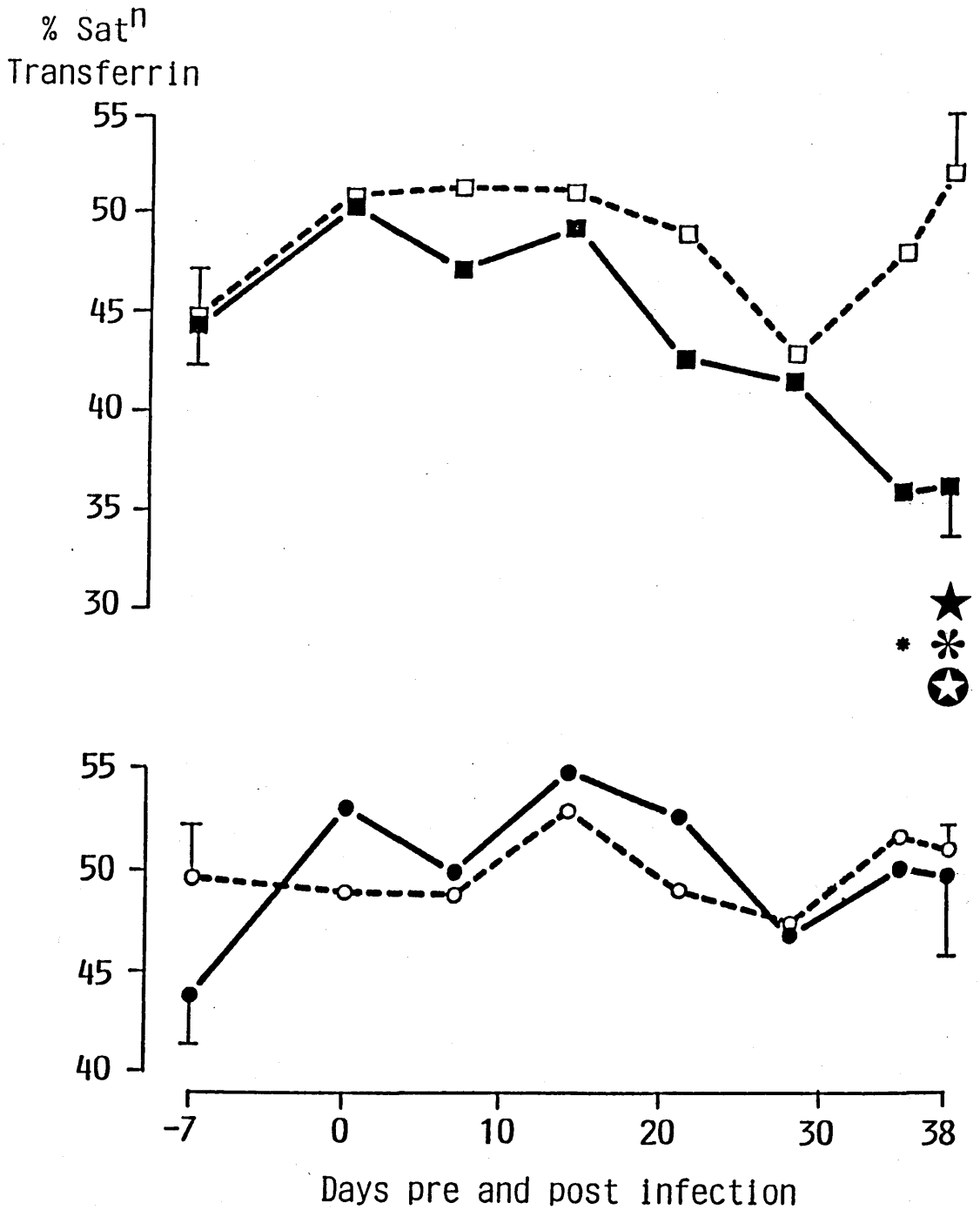

Figure 4.15 The percentage saturation (% satn) of transferrin of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□- - -□ ; ○- -○).

Significant effects:

Infection	★	p<0.01
Feeding level	*	p<0.05
"	✻	p<0.01
Interaction	✪	p<0.01

Table 4.6

Pathophysiological studies 25 - 38 days after infection (DAI). Haematocrit values (PCV); red cell (RCV), plasma (PV) and blood (TBV) volumes; red cell half-lives ($T_{\frac{1}{2}}$) and red cell (RBC) losses into the gastrointestinal tract (GIT) of lambs infected with 350 H. contortus larvae/kg bodyweight (BWt) and given a high (HFI) or low (LFI) plane of nutrition; and their respective pair-fed controls (HFC; LFC). Results are x (± SE).

Group	n	PCV (l/l) 25 DAI	RCV (^{51}Cr) ml/kg BWt	PV (^{125}I) ml/kg Bwt	TBV ml/kg BWt	$T_{\frac{1}{2}}$ RBC (^{51}Cr) h	RBC loss into GIT (^{51}Cr) ml RBC/d	Blood loss per parasite ml
HFI	3	0.240 (0.0104)	15.5 (1.48)	49.6 (3.36)	65.1 (4.69)	124.5 (12.46)	40.1 (10.46)	0.047 (0.0094)
HFC	3	0.310 (0.0161)	21.9 (0.15)	51.2 (2.98)	73.1 (3.13)	227.5 (11.09)	1.8 (1.12)	-
LFI	3	0.227 (0.0159)	13.5 (1.28)	46.6 (1.16)	60.0 (2.43)	113.7 (15.85)	32.4 (4.90)	0.044 (0.0054)
LFC	3	0.317 (0.0249)	20.7 (1.65)	48.5 (1.12)	69.2 (1.33)	283.7 (10.84)	0.4 (0.14)	-
Significant effects								
Feeding (F)								
Infection (I)		**	**		*	**	**	
F x I interaction						*		
Groups significantly different at an overall significance of P < 0.05						HFI < HFC LFI < LFC	HFI > HFC	

* P < 0.05
** P < 0.01

significantly greater in infected sheep than in controls. The mean loss was larger in group HFI than LFI, although not significantly so.

The uninfected sheep with urolithiasis, HFC-15, was losing 4 ml RBC/d into the GIT, equivalent to 12 ml whole blood per day (Appendix 2 Table 4). This sheep also lost more ^{51}Cr via the urine than any other sheep. Its urine ^{51}Cr activity was equivalent to 78 ml blood/d compared to 23 to 41 ml blood/d in the other sheep. The most likely explanation for this is a low grade haematuria associated with the cystitic change.

In the infected sheep the volume of whole blood lost per parasite per day ranged from 0.028 to 0.058 ml/d (mean 0.046), with little difference evident between the feeding groups. There was a significant positive correlation between the total worm burden (x) and the blood loss into the GI tract (y) ($y = 0.057x - 38.7$, $r = 0.854$, $p<0.05$).

Ferrokinetics (Table 4.7 and Appendix 2 Table 5)

Serum Fe concentrations were significantly lower in the infected sheep than in the controls.

The half-life of iron in plasma, measured from ^{59}Fe data, was significantly reduced by infection and was shorter, although not significantly so, among group LFI than group HFI (38 and 53 minutes respectively, compared to 117 and 119 in the control groups).

Group LFI had the highest mean plasma iron turnover rate, 306 umol/kg BWt per day, which was significantly greater than those of groups LFC (122) and HFI (222). The value for group HFI was higher than that of its control group HFC (155), but not significantly so. Thus, infection afforded a significant effect, and the F x I interaction was also significant.

Table 4.7

Ferrokinetic studies, 25 - 38 days after infection. Group mean (± SE) serum iron (Fe), plasma Fe half-life ($T\frac{1}{2}$) and the rate of plasma iron turnover (PIT) of lambs infected with 350 H. contortus larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition; and their respective pair-fed controls (HFC; LFC).

Group	n	Serum Fe µmol/l	^{59}Fe $T\frac{1}{2}$ mins	PIT µmol/kg Bwt/day
HFI	3	22.8 (3.56)	52.8 (13.22)	222 (24.0)
HFC	3	34.3 (1.45)	117.2 (13.38)	155 (26.4)
LFI	3	25.3 (1.30)	38.4 (1.99)	306 (14.9)
LFC	3	29.3 (1.09)	118.8 (12.14)	122 (15.0)
Significant effects				
Feeding (F)				
Infection (I)		**	**	**
F x I interaction				*
Groups significantly different at an overall significance level of P < 0.05			LFI < LFC	LFI > HFI, LFC

* P < 0.05
** P < 0.01

The percentage red cell utilisation of ^{59}Fe is shown in Figure 4.16 and was markedly affected by infection but not by feeding level. The % RCU peaked at 79 % in both groups HFI and LFI, four days post injection. In contrast, in both control groups the % RCU was much lower, reaching 57 % in HFC and 55 % in LFC by 12 days post injection.

Faecal Hb-Fe loss, measured with ^{51}Cr, averaged 816, 36, 692 and 11 mmol/d in groups HFI, HFC, LFI and LFC (Table 4.8 and Appendix 2 Table 6). Values measured with ^{59}Fe data were, with one exception, higher, and there was therefore no evidence of reabsorption of Hb-Fe in the GI tract.

Albumin metabolism

As mentioned previously, the infected sheep were significantly hypoalbuminaemic relative to controls. Both groups of infected sheep had significantly shorter albumin half-lives than their respective controls (Table 4.9 and Appendix 2 Table 7). Mean values were 189 and 220 h in HFI and LFI compared to 320 and 392 h in HFC and LFC. When the effect of nutrition was considered, both infected and control HF sheep had shorter half-lives than their respective LF groups, but the differences were not significant.

The intravascular albumin pool (CA) was significantly smaller in both groups of infected sheep (1.47 and 1.36 g/kg BWt in groups HFI and LFI) compared to their controls (1.81 and 1.71 respectively).

Similarly, extravascular pools (EA) were significantly depleted by infection, the group mean values being 1.79 and 2.78 g/kg BWt in groups HFI and HFC and 1.67 and 2.27 in groups LFI and LFC. Total albumin pools were also significantly reduced in the infected sheep. Group mean CA, EA and TA values were smaller among the LF groups

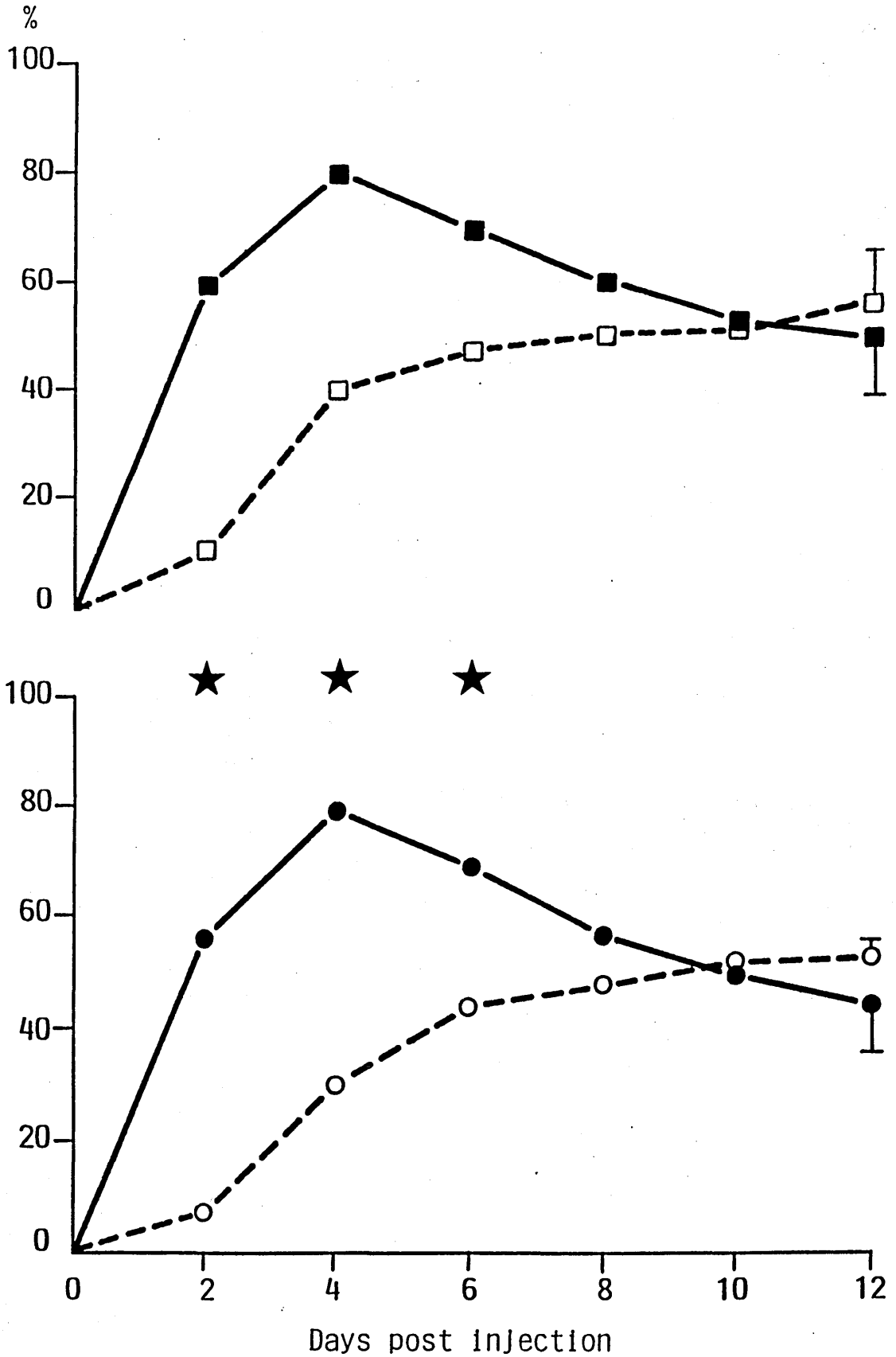

Figure 4.16 The percentage (%) utilisation of ^{59}Fe by red blood cells of lambs infected with H. contortus and given a high (■——■) or low (●——●) plane of nutrition, and their respective pair-fed controls (□- - -□; ○- - -○).
Significant effects: Infection ★ $p < 0.01$

Table 4.8

Ferrokinetic studies, 25 - 38 days after infection, Group mean (± SE) daily gastrointestinal (GI) loss and reabsorption of haemoglobin iron (Hb - Fe) in lambs infected with 350 *H. contortus* larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition; and their respective pair-fed controls (HFC; LFC).

Group	n	^{51}Cr		^{59}Fe		Intestinal Reabsorption of Hb - Fe µmol/d
		GI blood loss ml/d	Hb-Fe loss µmol/d	GI blood loss ml/d	Hb-Fe loss µmol/d	
HFI	3	181.7 (60.83)	816.0 (188.31)	190.7 (68.55)	851.7 (218.71)	-38.7 (29.23)
HFC	3	5.3 (3.38)	35.7 (23.92)	23.7 (5.21)	155.0 (39.46)	-119.3 (23.18)
LFI	3	152.7 (31.15)	691.7 (113.38)	164.7 (31.97)	746.3 (115.52)	-54.7 (13.32)
LFC	3	1.7 (0.33)	10.7 (2.03)	26.7 (7.26)	179.3 (57.62)	-168.7 (59.60)
Significant effects						
Feeding (F)						
Infection (I)		**	**	**	**	*
F x I interaction						

* $P < 0.05$
** $P < 0.01$

Table 4.9

Albumin metabolism studies, 25 - 38 days after infection. Group mean (± SE) serum albumin concentration; intravascular (CA), extravascular(EA) and total (TA) albumin pools, and the EA/CA ratio; plasma albumin half-life ($T\frac{1}{2}$) and the fractional rate of plasma albumin catabolism (k) of lambs infected with 350 H.contortus larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition; and their respective pair-fed controls (HFC; LFC).

Group	n	Serum Albumin g/l	CA g/kg BWt	EA g/kg BWt	TA g/kg BWt	EA/CA	$t\frac{1}{2}$ ^{125}I h	k
HFI	3	29.8 (1.42)	1.47 (0.029)	1.79 (0.041)	3.27 (0.060)	1.22 (0.026)	189 (16.2)	0.164 (0.0208)
HFC	3	35.3 (0.44)	1.81 (0.084)	2.78 (0.197)	4.59 (0.115)	1.56 (0.182)	320 (21.4)	0.109 (0.0183)
LFI	3	29.2 (1.74)	1.36 (0.079)	1.67 (0.074)	3.02 (0.119)	1.23 (0.084)	220 (30.9)	0.149 (0.0199)
LFC	3	35.2 (1.64)	1.71 (0.104)	2.27 (0.090)	3.98 (0.176)	1.33 (0.064)	392 (35.6)	0.091 (0.0018)
Significant effects								
Feeding (F)				*	**			
Infection (I)		**	**	**	**		**	*
Groups significantly different at an overall significance level of $P < 0.05$				HFI < HFC	HFI < HFC LFI < LFC			

* $P < 0.05$
** $P < 0.01$

compared to their respective HF groups, and this feeding level effect was significant with respect to TA and EA.

Albumin distribution was slightly affected by infection, with the ratio EA/CA showing a non-significant reduction in the infected groups relative to their controls.

The fractional rate of albumin catabolism (k) was significantly increased in the infected sheep (mean 0.156) compared to all the controls (0.100).

The uninfected sheep with urolithiasis, HFC-15, had a high k value (0.145), although the other parameters of albumin metabolism were not affected (Appendix 2 Table 7).

4.3.7 Carcase evaluation

Indicator joint dissection

The weight of the indicator joint was significantly greater in the HF groups than among the LF sheep but no effects of infection were apparent in the weight or composition of the joint (Table 4.10 and Appendix 2 Table 8). When comparing the experimental groups with group IC, it was found that the mean joint weights of groups LFI and LFC were significantly heavier, whereas those of groups HFI and HFC, although heavier still, did not register as statistically different, because of the greater variability in weight between joints within each group. This was due to inaccurate preparation of some of the joints, and largely negated the validity of this particular aspect of the experiment.

Body composition

Feeding level significantly affected bodyweight, empty bodyweight, total body protein and total body water, among both infected and control sheep (Table 4.11 and Appendix 2 Table 9).

Table 4.10

Mean (± SE) weight and composition of the right best-end neck joint of sheep infected with 350 *H. contortus* larvae/kg bodyweight, 42 days previously and given a high (HFI) or low (LFI) plane of nutrition and their respective controls pair-fed (HFC; LFC) or killed at the start of the experiment (IC).

	n	Jt Wt (g)	% bone	% fat	% total lean	% eye muscle	DM coeff. eye muscle
IC	8	256 (13.2)	27.6 (1.25)	17.1 (1.36)	55.3 (1.59)	24.3 (1.31)	0.243 (0.0027)
HFI	3	390 (25.8)	25.2 (1.46)	21.6 (0.86)	53.2 (2.21)	21.7 (1.66)	0.254 (0.0039)
HFC	3	344 (39.6)	23.6 (4.17)	22.0 (3.87)	54.3 (0.39)	23.8 (1.47)	0.251 (0.0066)
LFI	4	311 † (9.2)	25.3 (2.60)	20.4 (2.66)	54.2 (2.45)	23.5 (2.26)	0.238 (0.0065)
LFC	4	321 † (13.4)	26.0 (1.97)	22.0 (2.12)	51.9 (0.90)	22.5 (1.08)	0.247 (0.0052)

Significant effects
Feeding (F) *
Infection (I)
F x I Interaction

* $P < 0.05$
† Different from IC; overall significance of $P < 0.05$

Table 4.11

Body composition of lambs infected with 350 *H. contortus* larvae/kg bodyweight 42 days previously and given a high (HFI) or low (LFI) plane of nutrition, control lambs slaughtered at the commencement of the experiment (IC) and pair-fed controls (HFC; LFC). Figures are mean (± SE).

Group n	IC 8	HFI 3	HFC 3	LFI 4	LFC 4	Significant effects Feeding (F)	 Infection (I)	 F x I Interaction
Bodyweight (kg)	31.8 (1.71)	41.2 † (1.45)	38.0 † (0.58)	36.8 (0.97)	36.0 (1.77)	*		
Empty Body-weight (kg)	24.8 (1.28)	31.8 † (1.18)	29.1 (0.83)	28.3 (0.47)	28.3 (0.80)	*		
Total Body Fat (kg)	3.60 (0.228)	5.33 † (0.098)	4.94 † (0.079)	4.71 † (0.0055)	5.18 † (0.293)			*
Body water (% in fat-free empty body)	75.1 (0.44)	74.5 (0.52)	74.0 (1.01)	75.6 (0.35)	73.8 (0.86)			
Body protein (% in fat-free empty body)	19.3 (0.23)	19.6 (0.38)	20.5 (0.42)	19.3 (0.16)	20.0 (0.12)		**	
Total body protein (kg)	4.09 (0.237)	5.18 (0.22)	4.95 (0.141)	4.54 (0.055)	4.62 (0.130)	**		
Total body water (kg)	15.91 (0.790)	19.73 (0.907)	17.90 (0.825)	17.81 (0.335)	17.02 (0.445)	*	*	

* $P < 0.05$
** $P < 0.01$
† Different from IC; overall significance of $P < 0.05$

Infected sheep had significantly less body protein expressed as a percentage of the fat-free empty body, although no such effect was evident in total body protein. Infected sheep contained significantly more water, expressed as total body water, although when expressed as a percentage of the fat-free body, there was little difference between the groups. An interaction between feeding and infection was seen in the total body fat content of the sheep, with this being greater in the infected sheep given the high plane of nutrition, compared to their controls, but lower in the infected sheep sheep given the low plane of nutrition compared to their controls.

Considering the calculated gains over the course of the experiment (Table 4.12 and Appendix 2 Table 10), feeding level had a significant effect on bodyweight, protein and water gains. An interactive effect was observed in energy gain, with group LFI gaining significantly less energy than any other group.

When the composition of the net gain in empty bodyweight was examined (Table 4.13 and Appendix 2 Table 11), it was found that infection resulted in lower concentrations of fat and protein, and lower energy values. Additionally, sheep given the higher dietary level gained more protein and less fat per kg gain compared to those given the smaller ration.

Since the indicator joint data were considered unreliable, no attempt has been made to correlate that data with the body composition data obtained from the whole body studies.

Feed conversion (Table 4.14 and Appendix 2 Table 10)

The conversion of ingested dry matter to bodyweight gain (feed conversion) tended to be greater, but not significantly so, in the HF

Table 4.12

Group mean (± SE) gain in bodyweight (BWt), empty body, protein, fat, water and energy over 42 days in lambs infected with 350 *H. contortus* larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC).

Group	n	Gain (kg)					Gain (MJ)
		BWt	Empty body	Protein	Fat	Water	Energy
HFI	3	8.0 (0.50)	6.0 (0.47)	0.90 (0.100)	1.57 (0.070)	3.22 (0.506)	83.7 (2.67)
HFC	3	6.9 (1.09)	4.8 (1.20)	0.94 (0.175)	1.42 (0.043)	2.26 (1.090)	75.3 (3.84)
LFI	4	4.4 (0.32)	3.0 (1.19)	0.38 (0.070)	1.04 (0.077)	1.64 (0.081)	47.5 (5.38)
LFC	4	3.8 (0.66)	3.2 (0.42)	0.48 (0.036)	1.53 (0.313)	0.91 (0.174)	76.5 (11.99)
Significant effects							
Feeding (F)		**		**		*	
Infection (I)							
F x I interaction							*

* $P < 0.05$
** $P < 0.01$

Table 4.13

Composition of 1 kg net gain in empty bodyweight of lambs infected with 350 H. contortus larvae/kg bodyweight 42 days previously and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC). Figures are mean (± SE).

Group	n	Fat g/kg	Protein g/kg	Energy MJ/kg	Water g/kg
HFI	3	266 (33.3)	152 (13.4)	14.2 (1.69)	530 (49.3)
HFC	3	325 (60.4)	204 (20.8)	17.6 (4.03)	421 (104.7)
LFI	4	349 (11.1)	123 (16.1)	15.7 (1.59)	548 (35.0)
LFC	4	475 (51.4)	154 (11.0)	24.0 (1.02)	303 (59.2)
Significant effects					
Feeding (F)		*	*		
Infection (I)		*	*	*	
F x I interaction					

* $P < 0.05$

Table 4.14

The mean (± SE) feed conversion and apparent retention (%) of dietary energy and crude protein over 42 days by lambs infected with 350 *H. contortus* larvae/kg bodyweight and given a high (HFI) or low (LFI) plane of nutrition, and their pair-fed controls (HFC; LFC).

Group	n	Feed conversion BWt gain/ DM intake	Energy retention Δ TB Energy/ GE intake	Protein retention Δ TB Protein/ CP intake
HFI	3	13.4 (0.91)	7.9 (0.38)	10.4 (1.07)
HFC	3	11.3 (1.69)	7.1 (0.32)	10.8 (2.16)
LFI	4	10.7 (0.78)	6.6 (0.76)	6.4 (1.16)
LFC	4	9.2 (1.57)	10.7 (1.65)	8.0 (0.59)
Significant effects				
Feeding (F)				*
Infection (I)				
F x I interaction			*	

* $P < 0.05$

BWt Bodyweight
TB Total body
GE Gross energy
CP Crude protein

groups than the corresonding LF groups, and also in the infected sheep compared to the controls.

With regard to the mean apparent retention of dietary energy, a significant F x I interaction was observed whereby group LFI retained least (6.6 %) of the dietary energy, their uninfected controls most (10.7 %), and the two groups given the higher feeding level showed an intermediate retention with no apparent effect of infection (7.9 and 7.1 % respectively in groups HFI and HFC).

Considering the retention of dietary crude protein, a significant feeding effect was recorded, with the HF sheep retaining considerably more than their LF counterparts (10.4 and 10.8 % in groups HFI and HFC, compared to 6.4 and 8.0 % in LFI and LFC). The values for the infected sheep were slightly lower, particularly among the LF sheep, but no statistical significance was attached to these differences.

4.4 Discussion

As in the experiment described in the previous section, infection of young cross-bred lambs with 350 H. contortus larvae per kg BWt resulted in the sheep developing a moderate anaemia, but with infection having no apparent effect on feed intake or bodyweight changes. However, in this experiment it was found that infection affected body composition and feed utilisation, and these effects were more marked when the lambs were maintained on a lower plane of nutrition. The radioisotopic studies confirmed previous observations of the profound effects of haemonchosis on blood protein metabolism, and demonstrated that altering the plane of nutrition added little to these pathophysiological changes.

With the exception of the three animals which developed clinical urolithiasis, the lambs grew and ate well throughout the study. The effect of housing in metabolism stalls was again apparent. Voluntary feed intake was reduced for the first few days and gains in bodyweight were reduced or zero over this whole period. This was despite the sheep being accustomed to living in individual pens, from which it seemed a small step to the greater constraint of a metabolism stall. In addition, there were no outward manifestations of stress or discomfort to account for their reduced performance in the stalls. The sheep appeared to settle quietly within a few hours of introduction to the stalls, and quickly got used to the additional handling required for bag-changing and adjustments of harnesses. The sheep spent much of the day lying quietly, and the only period of excited activity was immediately prior to feeding in the morning. The explanation for the metabolism stall stress phenomenon remains unresolved, but it does remains a problem in experiments such as this

where the check on growth confounds the calculation of feed utilisation over what should otherwise have been a period of virtually constant growth. Since all the sheep experienced a similar check, the effect of cage-stress on feed utilisation has been ignored in this comparison between infected and control sheep.

Parasite establishment, as measured by the number of worms recovered at slaughter, was slightly lower among group HFI than the infected sheep offered the lower plane of nutrition. Overall, parasite establishment was similar to that seen in Experiment 3a, and in the corresponding infection of Abbott et al (1986a). The peak mean egg production at 30 DAI, when corrected for the difference in faecal mass, was similar in both groups. However, shortly after this peak, egg excretion from group LFI dropped sharply, and by the end of the experiment was less than half that excreted by the fewer worms harboured by group HFI sheep. In addition, there was no correlation between egg output and worm burdens of individual sheep. This highlights the limitation of faecal egg counts as a measure of the infection borne by individual sheep, a problem previously noted by other workers, for example in haemonchosis (Abbott, 1982) and bovine ostertagiasis (Entrocasso, 1984).

Small numbers of strongyle eggs, and a few H. contortus adults were recovered from some control sheep. From the experimental design, and the management practices employed, it seemed unlikely that the sheep would have become accidentally infected during the experiment. Since there was no pattern to the egg excretion (no two consecutive samples from any one sheep were both positive) the most likely explanation is that the samples were cross-contaminated, either at the time of collection, in the case of faeces samples, or

during preparation in the laboratory. Samples in this experiment were analysed 'blind', with the operator unaware of the infection status of the sheep. This reduced the risk of operator bias, but increased the chance of cross-contamination of samples. For future experiments it was considered preferable to group faecal samples into 'infected' and 'control' batches, to minimise contamination of negative samples with strongyle eggs.

The sheep given the lower feeding level tended to have higher coefficients of digestion than the HF groups, an observation which agrees with previous work summarised by ARC (1980). The reason why infected sheep offered the smaller ration should have higher digestibility coefficients than their controls, while the reverse situation occurred among the HF sheep, remains unclear. This finding, and the considerable individual variation recorded in nitrogen balance throughout the groups do, perhaps, highlight the limitations of such studies, especially where each treatment group comprises only a small number of animals. When the retention of dietary crude protein and gross energy was measured over the 42 day infection period, significant reductions were detected in infected compared to control sheep. Similarly, the net gain in empty body of the infected sheep over this period was shown to contain significantly less protein and gross energy than that of the control sheep. These findings, which would not have been predicted by the seven-day nutritional balance results, imply that either the effects of infection on nitrogen and energy retention were manifested at an earlier stage of infection, or that a seven day balance is not sufficiently sensitive to detect differences of the magnitude experienced in this experiment. The former hypothesis seems less

likely, since the changes in blood protein metabolism which appear to underly the pathophysiology of this infection were certainly evident at the time the nutritional balance studies were conducted.

The anaemia which developed in the infected sheep was of the normochromic, macrocytic character previously described by Dargie and Allonby (1975), although less severe than that typically found by previous workers (Abbott et al, 1985a; 1986a). In this study, the severity was not much influenced by the level of nutrition, and was similar to that seen in Experiment 3a. When the erythrokinetics and blood volumes were measured radioisotopically, it was found that, again, the plane of nutrition did not much affect the severity of the changes due to haemonchosis, although among the control sheep red cell half-lives were considerably longer in group LFC than in the sheep given the larger ration. Abbott et al (1986b) similarly reported that the level of nutrition did not affect the extent of the pathophysiological changes in lambs given 350 H. contortus/kg. However, in their earlier study of lambs given 125 larvae/kg (Abbott et al, 1985b), they found that in Finn Dorset, but not in Scottish Blackface lambs, infection was associated with greater red cell losses and shorter red cell half-lives in lambs given the low protein ration, compared to those given the higher protein diet.

It was interesting to record, as in the previous experiments, a reduction in white cell counts among the infected sheep compared to the controls. Since differential white cell counts were not performed, the character of the leukopenia remains unresolved.

A decrease in circulating albumin concentration, as recorded here, is a common finding in trichostrongylid infections (eg Holmes and Mclean, 1971; Steel et al, 1980; Abbott, 1982). In many

cases a simultaneous hyperglobulinaemia develops, and the total serum protein concentration may be little affected (Steel et al, 1980; Abbott, 1982). In this experiment, however, globulin values tended to fall, especially in group LFI, and the infected sheep showed a significant total hypoproteinaemia.

The data obtained from studying radio-labelled albumin agree with previous results of Abbott (1982). That is, infection with H. contortus causes a reduction in the half-life of albumin, and an increase in the rate of its catabolism. In this experiment, the half-life of albumin was a little shorter in the HF sheep compared to the LF groups, and in this respect the quantitative manipulation of nutrition practised here produced the same effect that Abbott (1982) created by altering the concentration of protein within the diets offered to the experimental sheep.

It was interesting that sheep HFC-15, which was inappetant and affected by sub-acute urolithiasis, also showed an increased fractional rate of albumin catabolism. The increased loss of ^{125}I in the urine of that sheep, accompanied by increased urinary ^{51}Cr, almost certainly resulted from a different mechanism to that active in the parasitised sheep, and probably related to a low-grade haemmorrhagic cystitis accompanying the urolithiasis.

Serum iron concentration and iron binding capacity were only markedly reduced in group HFI. However, using radio-iron, it was found that the half-life of plasma iron was slightly shorter in group LFI than in group HFI. This apparent discrepancy can be reconciled when it is remembered that the circulating level of plasma iron reflects the body's available iron reserves, while the half-life of the element in plasma is a measure of the rate of erythropoiesis, and

thus these two parameters may not be closely related. However, there is no obvious reason why the better-fed HFI sheep should show a reduction in plasma iron when their LFI counterparts remained normoferraemic.

There was no evidence of any reabsorption of Hb-Fe from the GI tract in the infected lambs, as measured by the double isotope tracer method of Roche et al (1957). This contrasts with the findings of Holmes and Maclean (1969), and Dargie and Allonby (1975) who reported some reabsorption of Hb-Fe in sheep suffering blood loss due to fascioliasis and haemonchosis respectively. As here, however, Abbott (1982) found little evidence of Hb-Fe reabsorption, but she found that the absorption of dietary Fe was increased in infected sheep and that iron deficiency only developed in severely inappetant animals.

Feeding level influenced the amount, and to a lesser effect, the composition, of gain in both infected and control sheep. The effects of infection were not quite so large, but nevertheless this study did demonstrate for the first time that haemonchosis affects the composition of body gain, and in particular that infected sheep gained less protein, fat and energy per unit gain in body tissue than uninfected, pair-fed controls. The effects of feeding level and infection appear to be not merely additive and at least in the case of the total gain in energy over 42 days, a significant interactive effect was noted with the infected sheep given the smaller ration retaining disproportionately little dietary energy.

The differences in body composition between the groups were due, in part, to relatively higher water contents in the infected, and also the low feed animals. Increases in body water have been

reported previously in trichostrongylosis, for example by Sykes and Coop (1976) who found an increase in water concentration in the fat-free empty body of sheep infected with T. colubriformis, and Entrocasso, Parkins, Armour, Bairden and McWilliam (1986b) who reported decreased dry matter coefficients in muscle samples from cattle infected with O. ostertagi. That these parameters of body water content were not significantly affected in the present study is probably a reflection of the mild parasitic effects and the small numbers of animals per group, rather than there being an entirely different pathophysiological process underlying these effects in the different host-parasite systems.

In the body gain studies it proved relatively less fruitful to compare the four experimental groups (HFI, HFC, LFI and LFC) directly with the initial control group IC, because of the small numbers per group, and the variation in initial bodyweight within each group. Instead it proved more useful to calculate initial body composition values for each experimental sheep, based on its initial liveweight and linear regression equations derived from data measured from individuals of group IC.

Dissection of the indicator joint yielded little useful information. A problem was encountered whereby it was found that a few carcases had not been split accurately along the mid-line, and therefore the spinous processes were either completely absent or present in entirety on the right half joint used for dissection. This inaccuracy affected the joint weight and, particularly, the percentage bone. In this experiment, the joints were prepared by butchers at a commercial abattoir, and were not all uniform in appearance. The problem of inaccurate cutting had not been foreseen

as previously the same butchers had cut very neat, uniform joints. Obviously outside labour, not under direct supervision of the experimenter, is a potential source of error in any experiment, and in an ideal situation would not be used. The problem was minimised for future experiments by ensuring that greater care was exercised in splitting the carcases, but undoubtedly precision could be further improved by using the whole joint (right and left halves) in future studies.

There seems little doubt from the results presented here, and from those of previous workers, that one of the major pathophysiological derangements underlying haemonchosis is altered protein metabolism, following abomasal haemorrhage caused by the haematophagic activity of the parasites. Recent work by Rowe, Nolan, de Chaneet, Teleni and Holmes (1988) studied digestion in sheep infected with H. contortus, or sham-parasitised by transferring appropriate volumes of blood from the jugular vein to the abomasum. Both groups became anaemic, but an important difference between the infected and sham-infected sheep was that in the former group much of the additional blood nitrogen found in the abomasum was converted to ammonia by the parasites, and the metabolism of this ammonia in the liver resulted in higher urea synthesis. Thus, in effect, the presence of the parasites increased the non-recoverable loss of amino acids into the gut and increased the protein requirements of the infected sheep. The results of Experiments 3a and 4 suggest that the potential detrimental effects on animal production can be largely overcome by a high nutrient intake.

SECTION 5

The effect of subclinical haemonchosis on energy metabolism in sheep.

Section 5

The effect of subclinical haemonchosis on energy metabolism in sheep.

5.1 Introduction

Energy is an essential dietary requirement for normal growth and production, and it may be hypothesised that alterations in energy metabolism may underly the reduced production observed in parasitised animals. In studying energy metabolism in this context, a useful approach is investigation of the partitioning of dietary energy in infected and uninfected animals.

A proportion of the gross energy (GE) of the feed is lost to the animal in the faeces (EF), in urine (EU) and in eructated methane (EM). Measurement of these losses allows the calculation of the metabolisable energy (ME) available to the animal by the equation:

$$ME = GE - (EF + EU + EM)$$

The ME content of a foodstuff is a close approximation to the energy available to the animal for metabolism, although it ignores the heat of fermentation and the metabolic products in the faeces.

A portion of the ME is lost as heat and part of this loss, known as the heat increment, represents an inevitable increase in heat production associated with the digestion, absorption and metabolism of the ingested ME. ME minus the heat increment equals the net energy (NE) which can be regarded as the proportion of the GE that is used by the animal for maintenance and production. The part of the NE which is used for maintenance is liberated as heat which, together with the heat increment, amounts to the total heat production.

It is apparent, then, that an increase in any of the above energy losses will reduce the amount of net energy available to the animal for production, and therefore measurement of the partitioning of dietary energy in parasitised animals may yield clues to the mechanisms which result in reduced production in these animals.

As discussed in previous sections, faecal energy is not usually increased in haemonchosis. Little data is available regarding urinary and methane energy losses associated with this infection.

The radioisotopic studies described previously have shown that sheep infected with H. contortus lose a large quantity of blood into the abomasum each day. Although the blood components are digested and reabsorbed in the intestine and not lost in faeces, it seems apparent that digestion and absorption of the leaked blood, and its resynthesis into blood proteins, must represent a considerable energy cost to the host, and this may be measurable as an increase in heat production.

There are no reports in the literature of the measurement of metabolic rates of sheep infected with H. contortus. MacRae et al (1982) measured energy metabolism of lambs infected with T. colubriformis and found that although digestibility of the ration was reduced in infected animals, neither heat production nor the efficiency of utilisation of absorbed ME were affected by the infection. This is in contrast to the findings of Sykes and Coop (1976) who reported that infection with T. colubriformis did not impair digestion, and although they did not measure the metabolic rates of their sheep, they calculated that the gross efficiency of utilisation of ME in the infected sheep was 50 and 45% lower than in ad libitum and pair-fed controls respectively.

In investigating the partitioning of dietary energy, measurement of GE of the feedstuff, faeces and urine are easily achieved by bomb calorimetry (see Section 2). Measurement of methane production, and the energy value of that loss, requires collection and analysis of exhaust gases from the animal.

Heat loss may be measured directly (direct calorimetry), although the equipment required to measure both sensible (conduction, convection and radiation) and insensible (evaporative) heat losses is complex and expensive. Alternatively, heat production may be calculated indirectly, by measurement of the exchange of respiratory gases. Since the heat liberated within an animal's body is derived from the oxidation of dietary substances, and such oxidation reactions are stoichiometric, heat production can be estimated from measurements of O_2 utilised and CO_2 released by the animal. This is termed indirect calorimetry.

The present experiment was conducted to measure the effect of infection with H. contortus on the partitioning of dietary energy of sheep using indirect calorimetry.

Experiment 5 The effect of a single moderate infection with *H. contortus* on the partitioning of dietary energy in sheep measured using indirect calorimetry.

5.2 Materials and methods

5.2.1 Experimental design

Nine, eight to ten month old Suffolk X Greyface wether sheep were used. Three sheep were given a single infection of 350 *H. contortus* larvae/kg BWt, a fourth 500 larvae/kg BWt. The other sheep were maintained as uninfected controls, four being pair-fed to individual infected animals. As only one calorimeter was available, the infections were staggered at fortnightly intervals so that each sheep entered the calorimeter chamber at the same stage of infection.

The sheep were maintained in standard metabolism cages until 25 DAI when they were moved to the calorimeter chamber for a three day adjustment period followed by a four day nitrogen and energy balance conducted concurrently with 4, 24-hour gas exchange measurements (28-31 DAI).

Bodyweights, faecal egg counts and haematological changes were measured regularly throughout the experiment. The infected sheep were killed 35 DAI and their abomasal worm burdens determined.

Two of the control sheep were later infected and, along with two uninfected sheep, subjected to similar measurements.

5.2.2 Feeding

The diet used in this experiment was the complete pelleted ruminant feed, Super Star Cubes, the analysis of which is provided in Section 3. Infected sheep were offered feed *ad libitum* until 10

DAI, after which time their intake was stabilised at 90 % of their previous level to minimise any refusals during the calorimetric study period. Intake by each control sheep was restricted to the amount eaten by its infected partner at each stage of the experiment.

Because of previous problems with copper toxicosis in similar sheep, all drinking water was supplemented with 0.08 g ammonium molybdate and 0.7 g sodium sulphate per litre.

5.2.3 Experimental infections

Infected sheep were given 350 or 500 H. contortus larvae per kg BWt, prepared and administrated as described in Section 2. Five infected sheep were given larvae of the same laboratory strain used in the previous experiments ('Glasgow' strain). Because of concern over possible low parasite establishment and low pathogenicity of this strain, one sheep, I-62, was given larvae kindly supplied by Dr. E. Munn of the AFRC Institute of Animal Physiology, Babraham, Cambridge. This strain of H. contortus was termed the 'Babraham' strain (see Section 5.3, Table 5.1).

5.2.4 Indirect calorimetry

The closed circuit indirect calorimeter used in this experiment was that described by Kelly (1977), but modified in the interim to include a rear-opening door to allow more convenient handling of experimental animals.

Briefly, the calorimeter system comprised a sealable animal chamber connected by a circuit of pipes to CO_2 and H_2O absorbers and an oxygen-filled spirometer. Air was circulated continuously through the system, and the utilisation of oxygen by the animal and absorption of CO_2 caused the pressure within the system to fall, and oxygen to be admitted from the spirometer.

The respiration chamber contained a feeding system operated by solenoid and a water bowl connected by a U-tube to an outside reservoir to supply water ad libitum without altering the volume of the system. Faeces was collected in a polythene bag attached to a standard sheep harness. Urine was collected in a pan below the weldmesh floor.

Two fans circulated air within the chamber, and a heat lamp connected through a thermostat maintained a temperature of 20°C within the system.

Gas was circulated through the system by means of a rotary compressor. The absorption train consisted of 5 metal tubes containing silica gel to absorb water vapour, two 20 l polythene bottles each containing 5 l of 50 % (w/w) KOH to absorb CO_2, and a further bank of three silica gel tubes to absorb water vapour carried over from the alkali bottles.

Oxygen was stored at atmospheric pressure over water in a 160 l capacity spirometer, and was drawn into the system by negative pressure. Once empty, the spirometer was designed to refill by means of electrically operated inlet and outlet switches connected to a relay. A counter was included in this circuit to record the number of times the spirometer had been filled, and the volume of oxygen within the spirometer could be read off a scale on the spirometer itself.

Representative samples of chamber air were collected in a rubber bag attached to a sampling valve incorporated in the system.

The increase in weight of the CO_2 absorber and subsequent H_2O absorbers gave the amount of CO_2 produced, and the oxygen admitted was measured from the spirometer counter and scale. The

composition of the chamber air at the beginning and end of the period was determined, and the volume of methane produced was calculated from the concentrations in these two samples. As the system is sensitive to alterations in atmospheric pressure and temperature, all gas volumes were standardised to n.t.p.

Prior to the study, the system was tested for leaks and repaired where necessary, and calibrated using gas dilution techniques.

Daily routine

It had been found that the longest practical period for operation of this closed circuit calorimeter containing a sheep was 24 h due to the lowering of the oxygen concentration by the accumulation of methane within the system. Therefore the chamber was opened for 20 minutes each day during which time the sheep was tended and samples taken.

The chamber was opened at 0900 h each morning following a set procedure.

Firstly, gas flow through the absorption train and the oxygen flow were stopped. A gas bag was then attached to the gas sampling valve and, after rinsing the bag three times, a gas sample was taken. The spirometer scale and counter readings were taken, and the atmospheric pressure and temperature were recorded. The water bowl was topped up and the consumption of water recorded. Wet and dry thermometers inside the chamber were read, and the pressure inside the chamber was measured.

The absorption train was removed and replaced by a pre-weighed train and the spirometer was filled.

The chamber was then opened and the urine pan emptied, rinsed and acidified with 150 ml of 0.05 N sulphuric acid. The faeces bag

was changed, the feeder loaded, and the wet bulb thermometer topped up.

The chamber lid was then replaced and sealed, the absorption train connected, and the circulation pump started. A gas sample was taken, and spirometer, atmospheric pressure and wet and dry thermometer readings recorded as before. With the circulation pump stopped, pressure inside the chamber was measured. The pump was restarted and the flow rate adjusted and oxygen supply connected by means of the appropriate valves. High risk leak joints, eg. potash bottle heads, were tested for leaks with soapy water and repaired immediately if necessary.

The removed silica gel tubes and potash bottles were weighed individually, and the increases in weight from the previous day calculated. The total weight of CO_2 absorbed was calculated as the increase in weight of the potash bottles and the last three silica gel tubes. The weight of water absorbed was the increase in weight of the first five silica gel tubes.

The silica gel tubes were regenerated by blowing hot air through them until the emergent air was about 90°C. The potash bottles were drained, rinsed and refilled with KOH solution. These components were then weighed and the train reassembled in preparation for the next determination.

Faeces and urine outputs were weighed and stored at 4°C for no more than 7 days prior to analysis.

Analytical techniques

Gas analysis was undertaken using a dedicated series of Hartmann and Braun (West Germany) gas analysers. Oxygen in chamber gas samples was determined using a paramagnetic gas analyser, and CO_2

and methane concentrations using infrared analysers.

All other analyses were performed using the methods described in Section 2.

Calculation of heat production

The daily heat production of the sheep was calculated from the oxygen consumption, methane and CO_2 production, and the average daily urinary N excretion.

The oxygen consumption was determined as the volume of oxygen drawn in from the spirometer, plus the decrease in the volume of oxygen in the chamber system calculated from gas analysis figures.

Carbon dioxide production was calculated from the volume of CO_2 absorbed, and the increase in volume of CO_2 in the chamber system, again determined from gas analysis.

The volume of methane produced over the period was calculated as the difference in methane concentration between the end and beginning samples, multiplied by the system volume.

All the gas volumes were corrected to litres at n.t.p. per 24 h using appropriate correction factors.

The mean urinary N excretion (g/d) was determined over the four day study period.

Daily heat production was calculated from these figures using the factors recommended by Brouwer (1965) in the following equation:

$$\text{Heat Production (kJ/d)} = 16.08\ O_2\ (l) + 5.02\ CO_2\ (l) - 2.17\ CH_4\ (l) - 5.99\ N\ (g)$$

Four daily determinations of heat production were carried out, and a mean value included in the energy balance equation.

Energy balance (energy retained or lost) was calculated as the difference between ME intake and heat production.

E retained = ME - heat

or

E retained = GE - (EF + EU + EM + heat)

where EM = 39.52 CH_4 (1).

Efficiency of utilisation of absorbed ME

As there was variation in energy intake among the sheep, and all the sheep were retaining energy, it was possible to regress ME intake against E retained, and hence calculate the efficiency of utilisation of metabolisable energy for gain (k_f) in each group. The data was first expressed as $MJ/kg^{0.73}$, ie. per unit metabolic bodyweight.

$$k_f = \frac{\text{gain } (MJ/kg^{0.73})}{\text{ME intake } (MJ/kg^{0.73})}$$

Extrapolation of the regressions to zero gain provided an estimate of the requirement of ME for maintenance (MEm).

5.2.5 Statistical analysis

Data were analysed using the paired t-test and Wilcoxon test for pair differences. Relationships between variables were analysed by calculating the correlation coefficient r, and differences between regressions were assessed by analysis of variance.

5.3 Results

5.3.1 Clinical and bodyweight changes

The sheep remained bright throughout the study and no adverse clinical signs were detected. All the sheep gained weight, although slight decreases in bodyweight were apparent in most of the sheep over the period of the chamber study (Figure 5.1).

5.3.2 Feed intakes

Feed intakes of individual sheep ranged from 1200 to 1800 g FM per day, and these intakes were matched by the pair-fed controls.

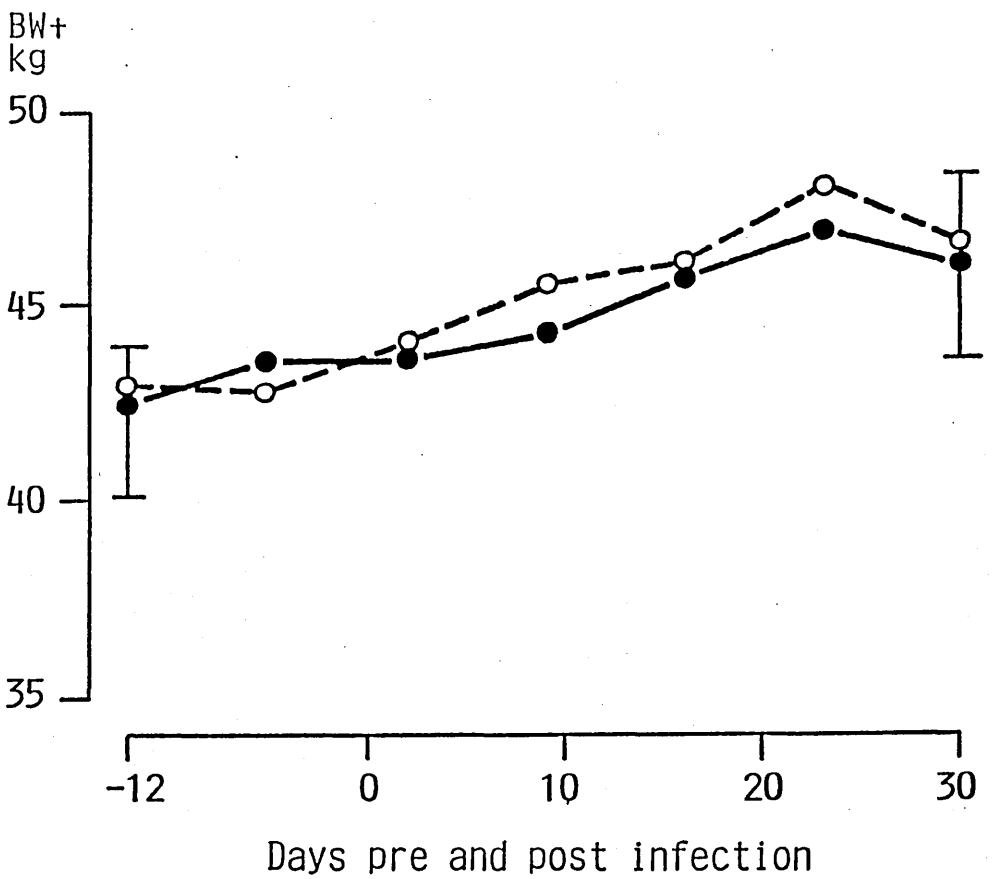

Figure 5.1 Bodyweight (BWt) of sheep infected with H. contortus (●——●) and uninfected pair-fed controls (O- -O).

During the chamber study small feed refusals (50 to 190 g/day) were evident in three infected and one control sheep.

5.3.3 Parasitological findings

Infections became patent between 16 and 23 DAI. Faecal egg counts were fairly low, the mean count peaking at 5000 epg at 23 DAI (Figure 5.2). The pattern and level of egg excretion varied between sheep, and individual peak egg counts are presented in Table 5.1.

Relatively small numbers of adult H. contortus, ranging from 247 to 2740 were recovered from the abomasa of the infected sheep at slaughter, representing 1.4 to 19.6 % of the larval challenge (Table 5.1).

There was a highly significant positive correlation between individual peak egg count and worm burden at slaughter ($r = 0.972$, $p < 0.005$), but no significant correlation between worm burden and egg counts on the day of slaughter ($r = 0.706$, $p > 0.1$).

5.3.4 Haematological and biochemical changes

Haematocrits fell slightly in response to infection (Figure 5.2) but differences between infected and control sheep were not significant at any time, and the values for each sheep remained at or above 0.3 l/l throughout the study. Values for other haematological parameters also remained normal throughout the infection.

Serum protein concentrations were not affected by infection, values for total protein, albumin and globulin remaining around 60, 36 and 24 g/l respectively in both groups throughout the infection period.

5.3.5 Nitrogen balance

There were no significant differences between the groups in N intake, faecal or urinary N loss or in N retention (Tables 5.2 &

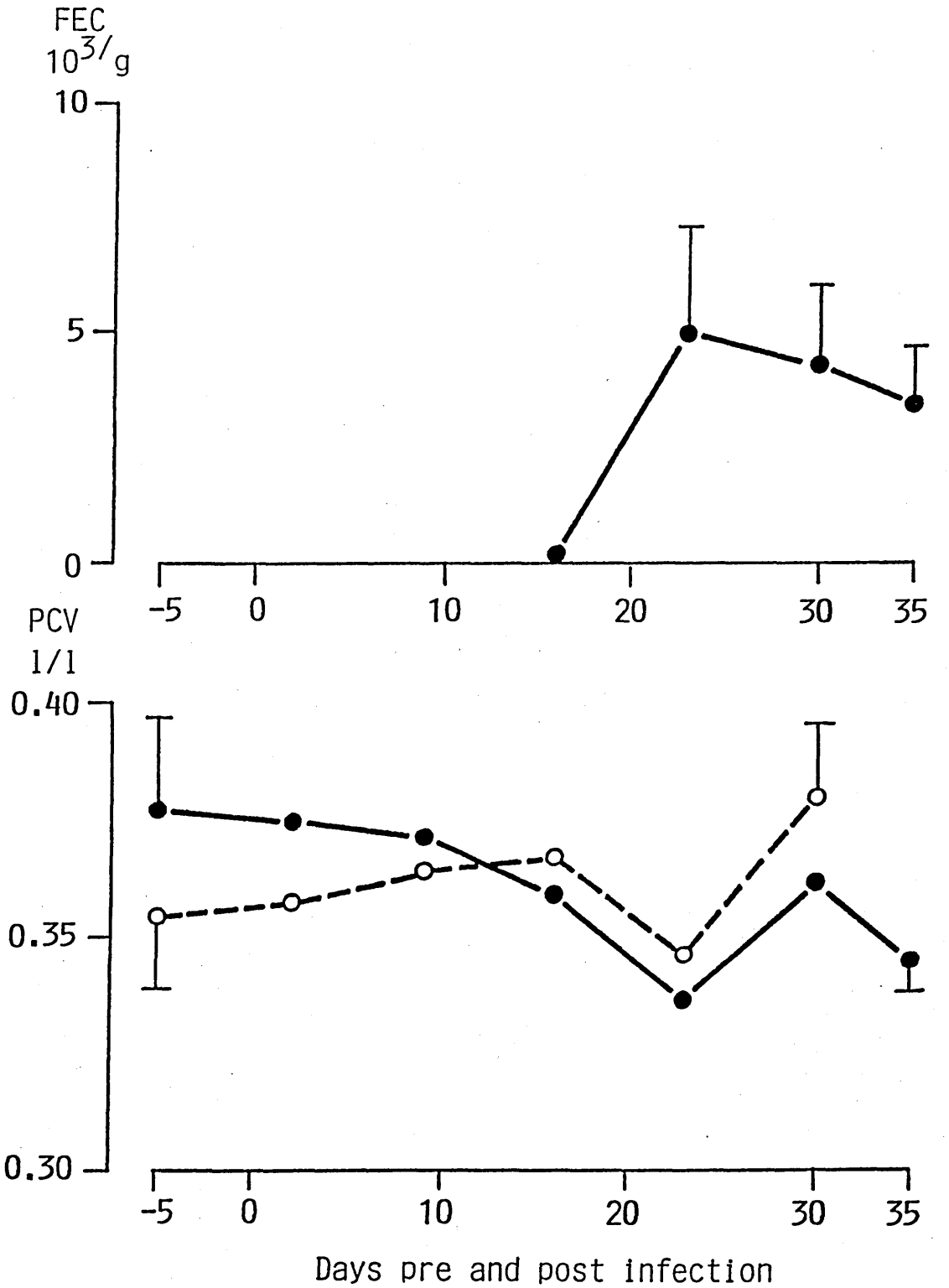

Figure 5.2 Faecal egg count (FEC) and packed cell volume (PCV) of sheep infected with H. contortus (●—●) and uninfected pair-fed controls (O--O).

Table 5.1

The larval challenge, worms recovered at slaughter, and peak strongyle egg excretion of sheep infected with *H. contortus*.

Sheep No.	*H. contortus*			Peak egg excretion	
	Larval Challenge[1,2]	Total Recovered at Slaughter	% Recovered	epg	DAI
I-53	21,000*	1,680	8.0	9,100	28
I-48	14,000	2,740	19.6	14,800	23
I-11	13,300	522	3.9	3,500	30
I-65	14,700	367	2.5	950	23
I-62	17,500†	1,876	9.3	7,050	23
I-67	17,500	247	1.4	1,600	35

1 350 larvae/kg BWt except:
* 500 larvae/kg BWT

2 Standard 'Glasgow' strain larvae except:
† 'Babraham' strain larvae

Table 5.2

Nitrogen (N) balance in sheep infected with H. contortus (I) and pair-fed controls (C).

Sheep No.	N g/d			
	Intake	Faeces	Urine	Retained
I-53	28.72	10.63	11.54	6.55
I-48	22.67	9.04	12.57	1.06
I-11	34.07	15.18	12.91	5.98
I-65	21.95	8.02	7.88	6.05
I-62	28.34	10.14	10.78	7.42
I-67	27.33	12.36	8.24	6.73
C-56	29.12	13.05	12.71	3.36
C-60	22.95	9.67	10.11	3.17
C-62	32.27	11.21	14.86	6.20
C-68	22.78	9.61	10.02	3.15
C-56	28.47	11.73	9.77	6.97
C-68	28.27	10.85	12.54	4.88

Table 5.3

Group mean Nitrogen (N) intake, faecal and urinary N losses, digestibility of N and N retention in sheep infected with *H. contortus* and pair-fed controls.

	Infected n = 6	Control n = 6	S.E.D.	Significance
N intake (g/d)	27.18	27.31	0.41	NS
Faecal N loss (g/d)	10.90	11.02	0.99	NS
Urine N loss (g/d)	10.65	11.67	0.99	NS
Digested N (g/d)	16.28	16.29	0.77	NS
Digested N (g/g N intake)	0.602	0.594	0.029	NS
N retention (g/d)	5.63	4.62	0.82	NS
N retention (g/g digested N)	0.341	0.279	0.055	NS

NS Not significant

5.3).

5.3.6 Energy balance

The partitioning of dietary energy in the two groups is presented in Tables 5.4 & 5.5.

Infected sheep produced more methane than controls, and lost on average 41 % more of their ingested gross energy in methane than did the controls. Statistical analysis showed this difference to be of borderline significance ($p = 0.05$).

Differences between the groups in faecal, urinary and heat losses were small, and as methane energy accounts for only a small proportion of ingested GE, overall energy retention was not significantly different between the groups.

Calculation of the efficiency of utilisation of ingested ME for gain (k_f) yielded values of 0.635 and 0.715 in the infected and control groups respectively. The calculated values of MEm were 0.459 (infected) and 0.515 $MJ/kg^{0.73}$ (control). These figures do not show statistically significant differences, and due to the small number of observations and the scatter due to individual variation it was not possible to determine from this data whether or not real differences due to infection did exist.

Table 5.4

The partitioning of dietary energy in sheep infected with H. contortus (I) and pair-fed controls (C).

Sheep No.	MJ/day					
	GE Intake	Energy in Faeces	Energy in Urine	Energy in Methane	Heat Produced	Energy Retained
I-53	23.63	8.42	0.68	0.69	8.75	5.09
I-48	18.69	6.85	0.73	1.06	7.75	2.30
I-11	28.43	12.34	0.86	0.71	10.44	4.08
I-65	17.64	5.94	0.47	0.94	8.52	1.77
I-62	23.65	7.74	0.77	0.74	10.59	3.81
I-67	22.73	7.94	0.63	1.15	10.49	2.52
C-56	24.30	8.63	1.00	0.62	9.63	4.42
C-60	19.16	7.15	0.67	0.47	8.22	2.65
C-62	25.43	8.50	0.79	0.79	10.41	4.94
C-68	19.01	7.33	0.74	0.56	9.04	1.34
C-56	23.76	9.23	0.83	0.56	10.63	2.51
C-68	23.60	7.39	0.80	0.94	10.04	4.43

Table 5.5

Group mean intakes of gross energy (GE), digestible energy (DE) and metabolisable energy(ME), the losses of energy in faeces, methane, urine and heat and the energy retention in sheep infected with H. contortus and pair-fed controls.

	Infected n = 6	Control n = 6	SED	Significance
GE Intake (MJ/d)	22.46	22.54	0.64	NS
Faecal Loss (MJ/d)	8.21	8.04	0.80	NS
(MJ/MJ GE)	0.362	0.358	0.02	NS
Methane Loss (MJ/d)	0.88	0.66	0.09	*
(MJ/MJ GE)	0.041	0.029	0.006	*
Urine Loss (MJ/d)	0.69	0.80	0.06	NS
(MJ/MJ GE)	0.031	0.036	0.003	NS
Heat Production (MJ/d)	9.42	9.66	0.19	NS
(MJ/MJ GE)	0.424	0.430	0.011	NS
Energy Retention (MJ/d)	3.26	3.38	0.47	NS
(MJ/MJ GE)	0.142	0.146	0.021	NS
DE (MJ/d)	14.26	14.51	0.39	NS
(MJ/MJ GE)	0.638	0.642	0.024	NS
ME (MJ/d)	12.69	13.04	0.38	NS
(MJ/MJ GE)	0.566	0.577	0.022	NS

* p = 0.05, Wilcoxon

N.S. Not significant

5.4 Discussion

The infected sheep maintained appetite and were only mildly affected by the infections as measured by faecal egg counts and haematological changes. Faecal and urinary energy losses were not affected by infection, nor was there any significant effect on heat production, calculated indirectly from gas exchange figures. Methane production was increased in the infected sheep. The data obtained for MEm and K_f in the two groups showed a trend whereby the infected sheep tended to have a lower requirement of ME for maintenance, and to show reduced efficiency of utilisation of energy for gain. The former result is surprising, the latter more predictable. However, no statistical significance could be attached to these results.

This experiment was not ideal in a number of respects, and the apparent lack of effect of infection on energy balance should not be taken as conclusive proof that no such effect might occur in natural infections. The main problems encountered here were an inexplicably poor parasite establishment in the infected sheep, and large within-group variation in the data rendering the experimental sample (six observations per group) too small for meaningful conclusions to be drawn.

Possible reasons for the lack of parasitic effect were investigated in a series of experiments described in Section 6, but no satisfactory explanation was obtained. Before any conclusions can be reached about the effects of haemonchosis on the partitioning of dietary energy, it would be useful to repeat this type of experiment using larger numbers of sheep, a greater range of dietary energy intakes, and ensuring that the sheep used were more severely

affected by the parasites.

However, despite the reservations expressed above, it was apparent in this experiment that the infected sheep converted a significantly greater proportion of dietary energy into methane than did their pair-fed controls. Methane production was fairly low in all the sheep, perhaps reflecting the nature of the feedstuff used, which had a low component of fermentable carbohydrate, and being chopped and pelletted, a short retention time in the rumen (ARC, 1980).

The mechanisms by which an abomasal parasite such as H. contortus affects methane production remain unresolved. It was not possible to differentiate between ruminal and caecal methane in this experiment as measurements were made in a whole body chamber.

An increase in methane from caecal fermentation would be a possible sequel to a situation where blood leaked into the abomasum passed through the small intestine and was subject to microbial fermentation in the caecum. However, a pilot study by Rowe et al (1982) using sheep fitted with duodenal and ileal cannulae showed that, at least in the case of nitrogen, material lost into the abomasum was reabsorbed from the gut lumen before the terminal ileum, and thus did not reach the caecum.

A more likely source of the extra methane is the rumen, since this is the major site of methane production in ruminants (Church, 1969), and this provokes the interesting hypothesis that the presence of parasites in the abomasum can in some way affect rumen function.

An increase in methane production would be compatible with either a total increase in rumen digestion, or an alteration in the pattern of rumen fermentation with a shift to production of shorter

chain volatile fatty acids (VFA) (Church, 1969). The first, a total increase in rumen digestion might be a sequel to increased retention time in the rumen, possibly mediated by alterations in motility. A change in rumen fermentation products might also accompany alterations in digesta retention time, and, or, possibly a change in the rumen microbial population.

Previous considerations of the effects of trichostrongylid parasites on digestive function have largely ignored the fore-stomachs, concentrating more on changes at, and distal to, the site of infection (see review by Gregory, 1985).

In sheep infected with T. colubriformis, Steel (1972) reported that ruminal acetate production was decreased, and Roseby (1977) recorded reduced rumen volume and outflow rates.

In haemonchosis, Bueno et al (1982b) found that placing larvae (sheathed, exsheathed or crushed) in the abomasum had no effect on reticular motility. When an extract of the crushed larvae was administered intravenously, reticular contractions were inhibited for a period of 40 to 60 minutes. However, this observation is probably of little relevance to natural infection.

Rowe et al (1988), in their study of digestion in sheep infected with H. contortus, reported a decreased ratio of acetate to propionate in rumen liqour (control 3.28; infected 2.58; SED 0.21; $p<0.05$), and an increase in rumen fluid outflow rate ($p<0.01$) from 4.05 l/d in the control group to 5.53 l/d in the infected group (SED 0.43). In addition, they recorded a decrease ($p<0.05$) in apparent digestion of organic matter in the forestomachs of infected sheep (0.32 compared to 0.39 in the controls; SED 0.02) and a similar decrease in apparent digestion of organic matter across the

whole digestive tract (0.65 control; 0.61 infected; SED 0.014; $p<0.05$).

Methane production was not measured directly, but both the shift in VFA ratio to propionate and the decrease in apparent digestion in the rumen would tend to suggest that methane production might have been decreased in this experiment.

In comparing the experiment of Rowe et al (1988) with the present study, it is difficult to reconcile the apparently opposite effects observed on rumen function. However, the experiments varied in many respects, such as the breeds of sheep, the diets and the levels of parasitism achieved. What the two experiments do suggest is that a more detailed examination of rumen function in sheep infected with H. contortus is justified and may add a further dimension to our understanding of the mechanisms responsible for production losses in parasitised animals.

SECTION 6

Some studies of factors influencing the pathogenicity of *Haemonchus contortus* in sheep.

Section 6

Some studies of factors influencing the pathogenicity of *Haemonchus contortus* in sheep.

6.1 Introduction

Over a period of months, several experiments were carried out which involved *H. contortus* infections in three- to ten-month old Suffolk x Greyface lambs. From the results it became apparent that an infective challenge of 350 *H. contortus* larvae/kg BWt caused marked anaemia in parasite-naive lambs at the beginning of this period (Section 3). However, some months later in the experiment described in Section 5, similar older lambs showed no such response to infection. In addition, faecal egg counts were lower than expected, and there was reduced recovery of parasites at slaughter.

A number of factors which might have been responsible were considered. Age-acquired resistance of the sheep, accidental previous exposure to parasite infection resulting in immunity, and the possible anthelmintic effect of medication with ammonium molybdate and sodium sulphate to prevent copper toxicity are factors which might have affected the susceptibility of the lambs to infection. Alternatively, the larval strain in use may have become attenuated either by repeated passage in culture sheep, or by storage in a refrigerator after harvesting from infected faeces.

That the sheep might have been accidentally exposed to parasitic infection during the pre-experimental housing period was considered unlikely. The lambs were maintained as a group in one pen without contact with any deliberately infected sheep, and no strongyle eggs

were detected in their faeces when sampled at intervals over this period. There was evidence of mild infections with Strongyloides papillosis and coccidia in some lambs, but it was deemed unlikely that this would have affected susceptibility to H. contortus.

The alternative hypotheses, that the larval strain may have become attenuated, that the molybdate/sulphate supplement may have had anthelmintic effects, or that the sheep may have developed an age-related resistance, were investigated in three experiments described here. In the first small study the pathogenicity of the suspect larval culture was compared to a strain whose virulence was not in doubt. In the second, lambs maintained for several months on various modifications of the molybdate supplement, or no supplement, were challenged with H. contortus (of the original strain) and the establishment of parasites in the various groups was compared. The third experiment measured the susceptibility of nine-month old sheep to infection with H. contortus, certain of the sheep having been previously vaccinated with radio-attenuated larvae.

Experiment 6a A comparison of the pathogenicity of two strains of *Haemonchus contortus* in Scottish Blackface sheep.

6a.1 Introduction

This experiment was designed to compare the pathogenicity of the suspect Glasgow strain of *H. contortus* with another strain of proven pathogenicity, kindly provided by Dr. E. Munn of IAP, Babraham, Cambs. This latter strain is hereafter referred to as the Babraham strain.

6a.2 Experimental design

Four, eight-month old Scottish Blackface ram lambs, purchased from a market in the West of Scotland where *H. contortus* is not a common parasite, were housed and treated with anthelmintic (fenbendazole, Panacur Sheep Wormer: Hoechst). Two weeks later, two of the lambs were infected with 10,000 *H. contortus* larvae. Lamb B-95 was given larvae of the Babraham strain, freshly cultured from faeces stored at room temperature for twelve days. Sheep G-96 was infected with Glasgow strain larvae, which had been harvested as above, and then stored for three months in a loose-topped jar at + 4°C. Two other sheep, C-93 and C-94, were maintained as uninfected controls (Table 6a.1).

At intervals after infection haematocrits and faecal egg counts were measured. The infected lambs were killed 35 days post infection, their abomasa removed and the parasites therein counted and identified.

Table 6a.1

Larval challenge and worm burdens at slaughter of sheep infected with different strains of *H. contortus* and uninfected controls (C)

Sheep No	Larval challenge	Worms recovered at slaughter			Worms Recovered (% larval challenge)
		Adult Male	Adult Female	Total	
B - 95	10,000 *H.contortus* Babraham strain	1,297	1,019	2,316	23.2%
G - 96	10,000 *H.contortus* Glasgow strain	2,950	2,556	5,506	55.1%
C - 93	Nil	-	-	-	-
C - 94	Nil	-	-	-	-

6a.3 Results

Haematocrit

Both infected lambs showed a marked drop in PCV from 0.37 l/l to 0.235 (B-95) and 0.165 (G-96). In contrast, PCVs of the two uninfected lambs remained above 0.36 l/l (Figure 6a.1).

Faecal Egg Counts

Nematode eggs were first seen in the faeces of B-95 at 22 DAI and of G-96 at 26 DAI. Faecal egg counts from G-96 were thereafter higher than from B-95, peaking at 15,900 and 7,600 epg respectively at 33 DAI (Figure 6a.1)

No nematode eggs were detected in the faeces of the control lambs throughout the experiment.

Worm Burdens

The abomasal contents of B-95 contained 2,316 adult H. contortus, with a male/female ratio of 1.27. The percentage of the original challenge recovered was 23.2%.

Sheep G-96 had 5,506 adult H.contortus in its abomasum, and the male/female ratio was 1.15. The percentage recovery of the original challenge was 55.1% (Table 6a.1).

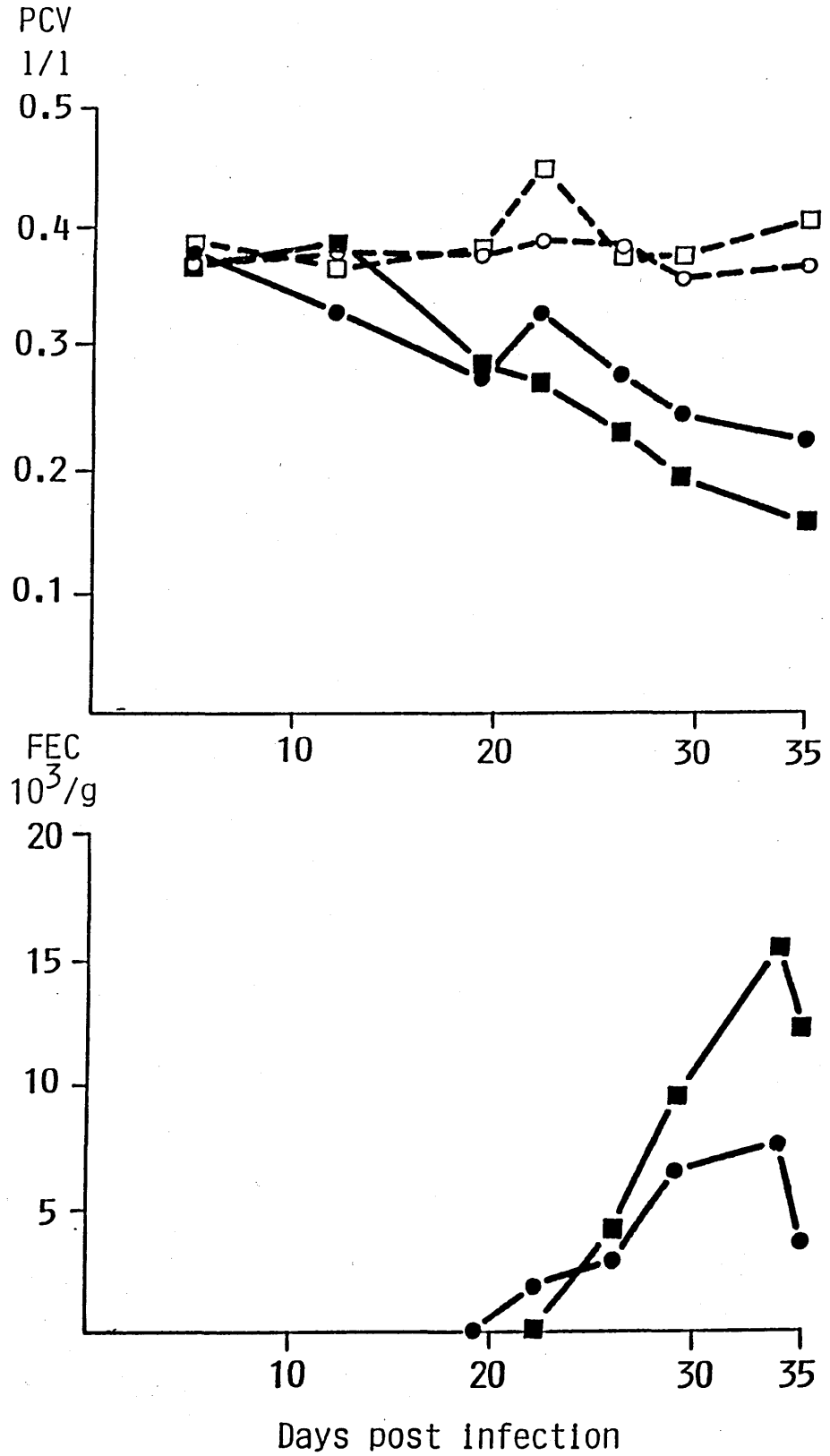

Figure 6a.1 Packed cell volume (PCV) and faecal egg count (FEC) of individual sheep infected with Glasgow (G-96, ■—■) or Babraham (B-95 ●—●) strain H. contortus and PCVs of uninfected control sheep P-93 (□---□) and P-94 (o---o).

Experiment 6b The effects of supplementation with sodium sulphate and ammonium molybdate on plasma and liver copper concentrations, and on the pathogenesis of ovine haemonchosis.

6b.1 Introduction

This experiment was conducted to examine the effect of medication with sodium sulphate and ammonium molybdate, administered to prevent copper toxicosis, on the pathogenesis of ovine haemonchosis.

It has long been recognised that the requirements of sheep for copper (Cu) are fairly critical, with clinical syndromes being associated with both deficiency and excess. The absorption and metabolism of dietary Cu are closely interlinked with levels of dietary molybdenum (Mo) and sulphur (S), and many cases of apparent Cu deficiency or toxicity are associated with normal dietary levels of Cu, and an excess or deficiency of Mo or S (Suttle, 1977). The biochemical mechanisms underlying this interdependence involve ruminal interaction of molybdate and sulphides giving rise to thiomolybdates which decrease the availability of dietary Cu and, if absorbed, impede the metabolism of tissue copper and inhibit copper enzymes (Mason, 1981).

Copper is a cumulative poison to sheep, excess absorbed Cu being stored in the liver. When the capacity of the liver to store Cu has been exceeded (approximately 1,000 mg Cu/kg DM liver), an increase in plasma Cu occurs, and an intravascular haemolytic crisis with haemoglobinuria and jaundice develops (Sharman, 1983).

Sheep most at risk are those maintained on systems in which

concentrate feeds form a high proportion of the diet for relatively long periods. The reason for this is the relatively high Cu content of cereal-based rations (10 - 20 mg/kg DM), and the high availability of that Cu due, in part, to low levels of Mo and S in cereal grains (Todd, 1972). It has been shown that the administration of supplemental Mo and S reduces the risk of Cu toxicity in sheep fed concentrate rations (Suttle, 1977).

Since Cu toxicity had proved to be a problem in similarly-reared lambs the previous year, a molybdate/sulphate supplement was administered to sheep in the experiment described in Section 5 of this thesis. However, adminstration of the supplement coincided with relatively low pathogenicity and parasite establishment. This experiment was therefore conducted to assess the effect of the original supplement, and variations of it, on the Cu status of growing lambs and on the establishment and pathogenesis of a single infection of H. contortus in these animals.

6b.2 Experimental design

Twenty, four-month old Suffolk x Greyface ewe lambs raised parasite-free from birth were divided into four groups of five animals to provide equal mean bodyweights in each group. Each group was maintained in a separate concrete pen on deep-litter straw bedding. The lambs were offered 500 g FM Super Star Cubes (see Section 4.2) each per day, and hay of medium quality ad libitum. The Cu content of the Super Star Cubes was 15.3 mg/kg DM. Unfortunately, the Cu content of the hay was not determined. Groups A, B and C received drinking water medicated with one of three supplements of ammonium molybdate and sodium sulphate, as shown in Table 6b.1. The supplements can be summarised as A, full

Table 6b.1

Composition of drinking water supplements given to sheep subsequently infected with *H. contortus*.

Group	Sheep No.	Supplement (g/l drinking water)
A	A-80	0.7 g sodium sulphate
	A-75	0.08 g ammonium molybdate
	A-70	
	A-79	"Full Supplement"
	A-62	
B	B-61	0.07 g sodium sulphate
	B-78	0.008 g ammonium molybdate
	B-63	
	B-68	"10% Supplement"
	B-69	
C	C-71	0.08 g ammonium molybdate
	C-50	
	C-67	"Molybdate only"
	C-66	
	C-72	
D	D-65	Nil
	D-76	
	D-77	
	D-74	
	D-64	

supplement; B, 10% supplement; and C, molybdate only. Group D was provided with ordinary tap water.

After two months, each sheep was infected with 350 *H. contortus* larvae/kg BWt and killed 33 DAI. Bodyweights, PCVs, plasma copper concentrations and faecal egg counts were measured regularly throughout the experiment. At slaughter, worm burdens were assessed, and the weight and copper content of the livers were determined.

The techniques used are all described in Section 2.

Analysis of variance was applied to the data, and differences between group means were further analysed using a multiple comparison procedure with Bonferroni correction.

6b.3 Results

Clinical and bodyweight changes

One sheep, A-80, developed clinical haemonchosis. It was the smallest lamb in the experiment, and did not grow much over the experimental period. It showed pale mucous membranes from 19 DAI, and submandibular oedema around 31 DAI. The other sheep grew well (Figure 6b.1) and showed no adverse clinical signs.

Faecal egg counts (Figure 6b.2)

The time at which eggs were first detected in the faeces of individual sheep ranged from 17 to 24 DAI. All the sheep in Group A excreted eggs 17 DAI, whereas of the other 15 sheep, only five (one or two per group) were positive at that time. Mean faecal egg counts from groups A and D peaked at 27 DAI, with values of 18,600 and 20,640 respectively. In groups C and B, mean values reached 15,750 and 41,180 by 32 DAI. The latter figure includes a count of 138,000 epg from B-78, the highest value recorded from any sheep in

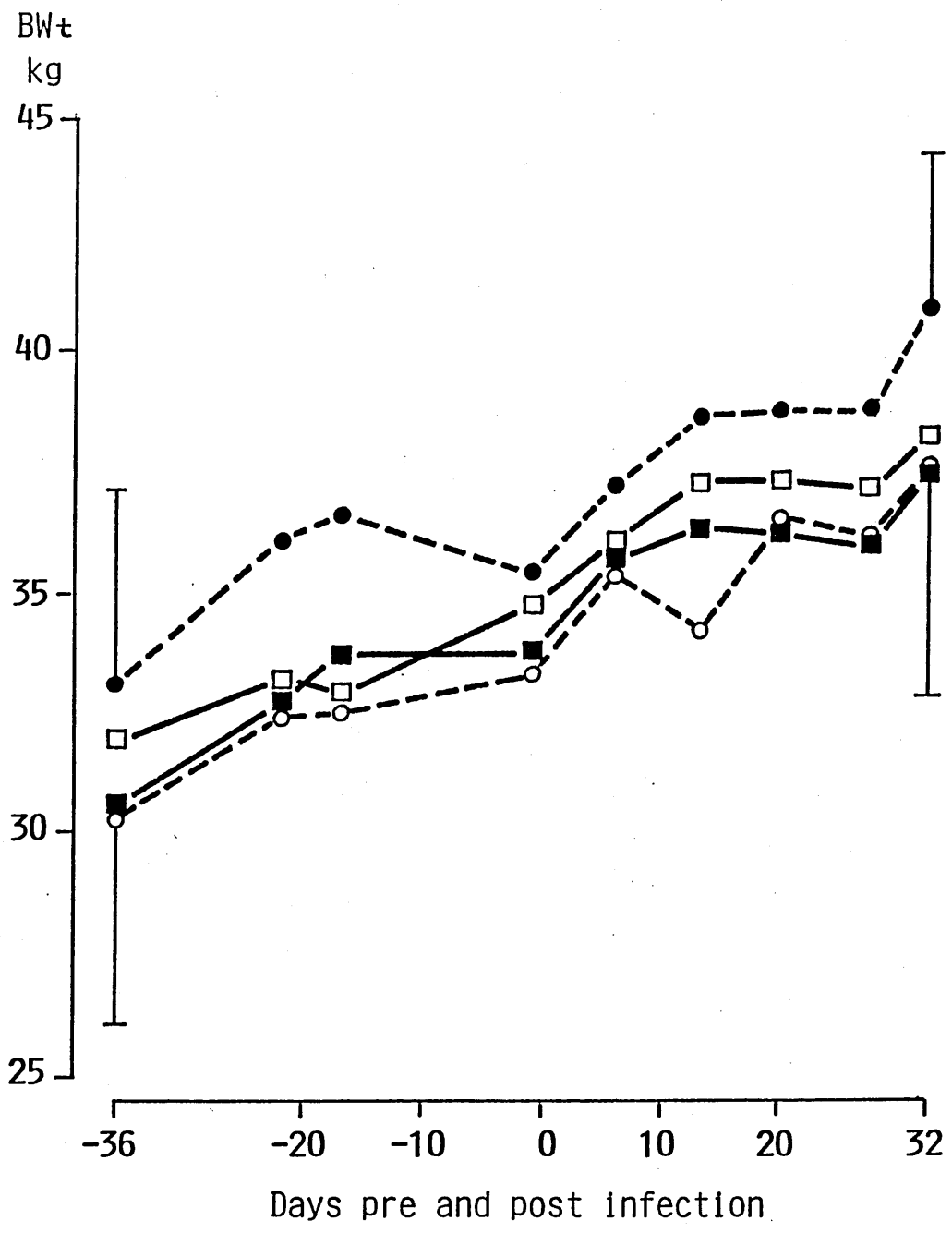

Figure 6b.1 Bodyweight (BWt) of sheep infected with H. contortus and given drinking water supplements, details of which are shown in Table 6b.1. Group A (■——■) full supplement, Group B (□——□) 10% supplement, Group C (●– – –●) molybdate only, Group D (O– – –O) no supplement.

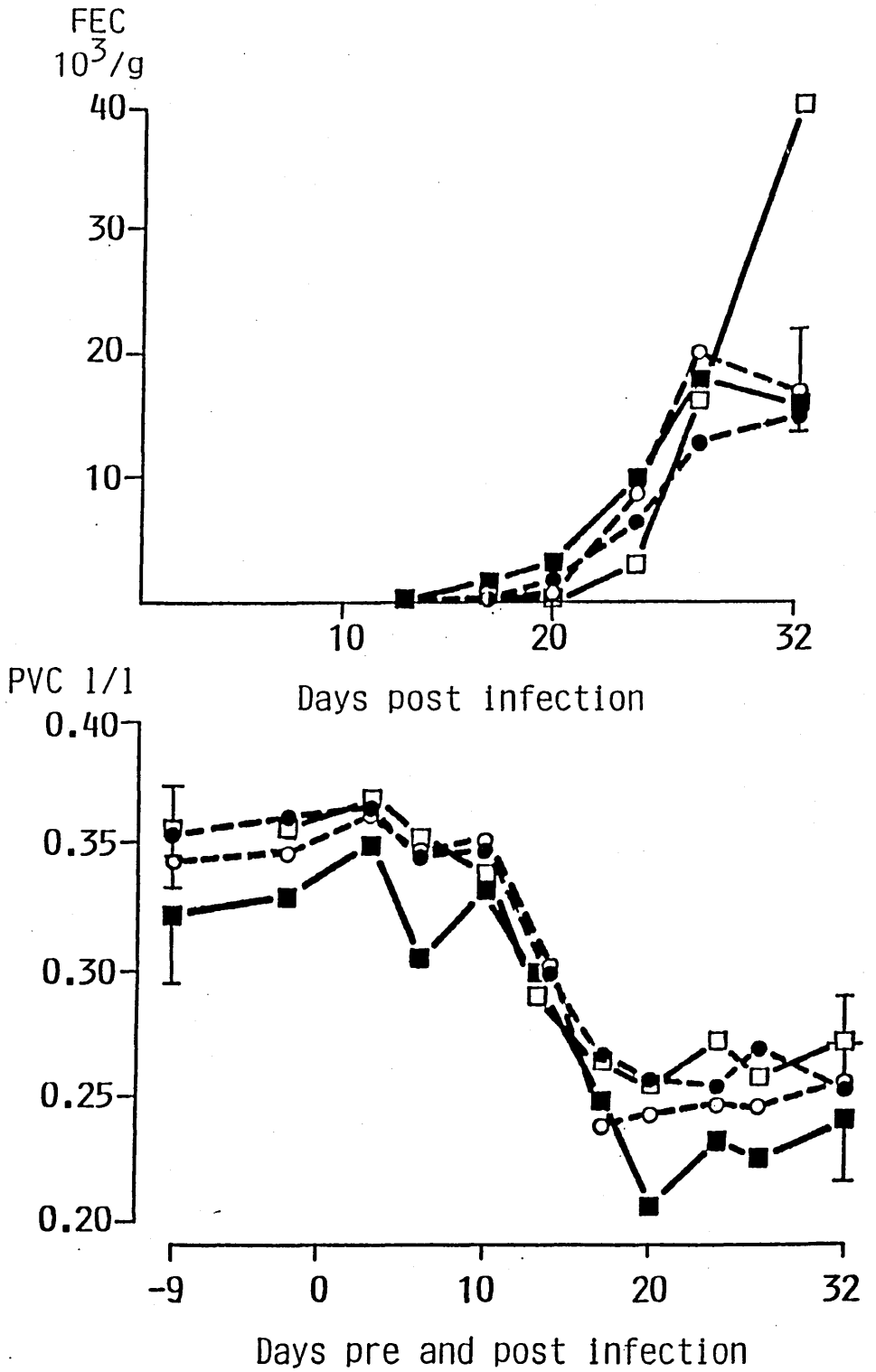

Figure 6b.2 Faecal egg count (FEC) and packed cell volume (PCV) of sheep infected with H. contortus and given drinking water supplements, details of which are shown in Table 6b.1. Group A (■———■) full supplement, Group B (□———□) 10% supplement, Group C (●——●) molybdate only, Group D (O——O) no supplement.

the experiment.

Worm burdens

The number of worms recovered from each sheep at slaughter is shown in Table 6b.2. The mean burdens for groups A, B, C and D were 6,926, 5,898, 4,676 and 6,436 respectively, with no significant differences between the groups.

Packed cell volumes

The mean PCV of each group fell sharply between 10 and 20 DAI, and then remained low until slaughter (Figure 6b.2). The largest drop in PCV was in group A, although the differences between the groups were not significant. The lowest individual value recorded was 0.16 l/l from sheep A-80, 27 DAI. Haematocrits of the other sheep remained at or above 0.20 l/l.

Serum protein concentration

The mean total serum protein concentration fell after infection from around 60 g/l, to about 50 g/l by 32 DAI (Figure 6b.3). Final values were slightly lower in groups A and B than in C and D because of very low concentrations in sheep A-80 (42 g/l, 32 DAI) and B-78 (43 g/l, 27 and 32 DAI). The four groups showed drops in both albumin and globulin concentrations over the infection period, with no significant differences between the groups.

Plasma copper concentration

These data are illustrated in Figure 6b.4 and Table 6b.3.

At the beginning of the experiment, sheep of groups A and C had significantly higher Cu concentrations than those of groups B and D (1.68 and 1.75 ug/ml in A and C; 1.08 and 0.96 in B and D). Small increases were observed in the mean values of groups A, B and D in the pre-infection period, and in group A during the infection by the

Table 6b.2

Larval challenge and worm burdens at slaughter of sheep infected with *H. contortus* and given drinking water supplements, details of which are provided in Table 6b.1.

Group	A	B	C	D	Significance
Supplement	Full Supplement	10% Supplement	Molybdate only	No Supplement	
Larval challenge	11,830 (1,744)	12,180 (1,382)	12,390 (1,081)	11,690 (1,441)	NS
Total worms recovered	6,926 (969)	5,898 (979)	4,676 (573)	6,436 (661)	NS
Male/Female	0.81 (0.03)	0.87 (0.05)	0.81 (0.08)	0.89 (0.14)	NS
Worms recovered (% challenge)	59 (2.6)	48 (6.5)	40 (8.0)	56 (2.1)	NS

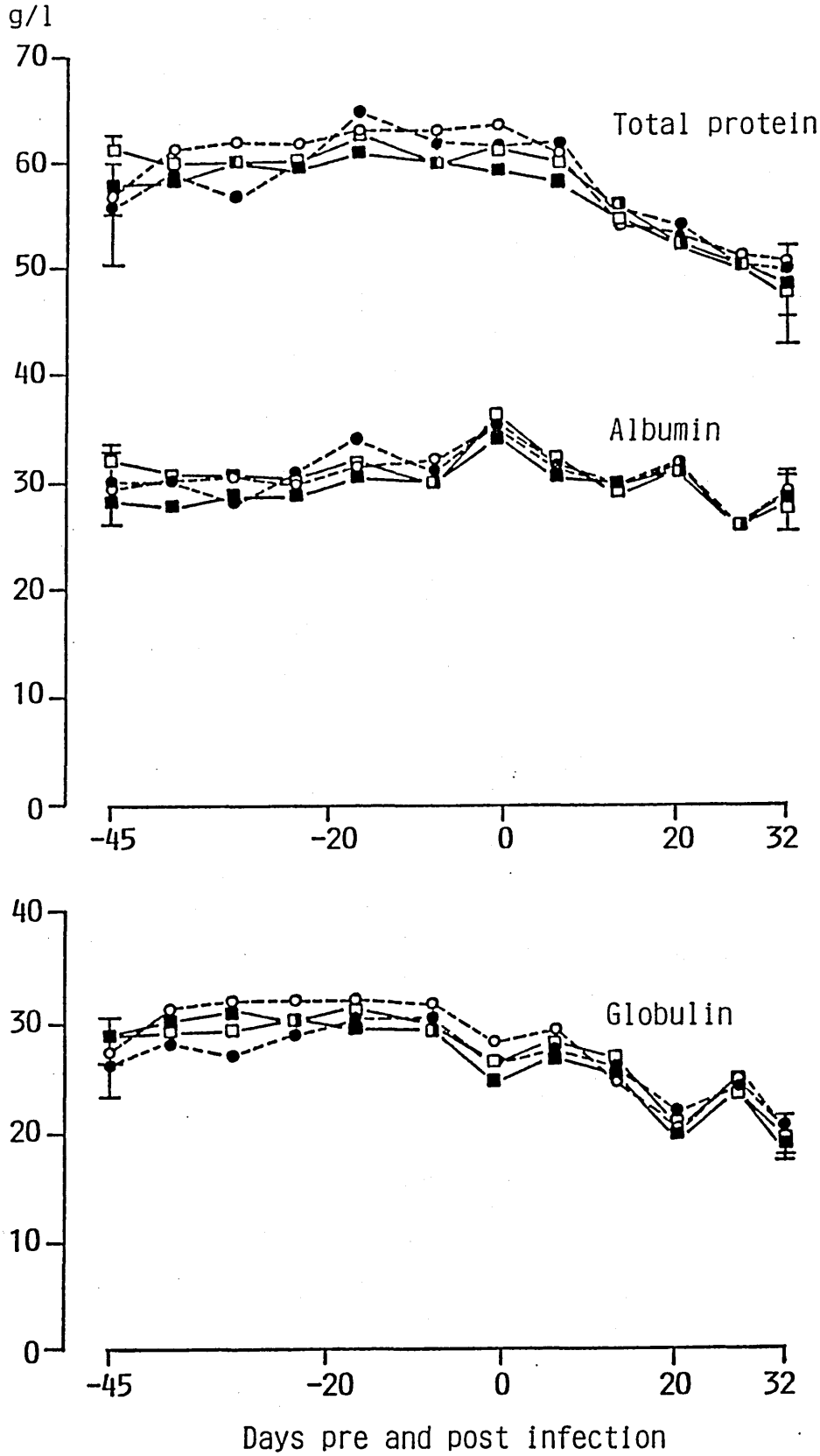

Figure 6b.3 Serum total protein, albumin and globulin concentrations of sheep infected with H. contortus and given drinking water supplements, details of which are shown in Table 6b.1. Group A (■——■) full supplement, Group B (□——□) 10% supplement, Group C (●— —●) molybdate only, Group D (O— —O) no supplement.

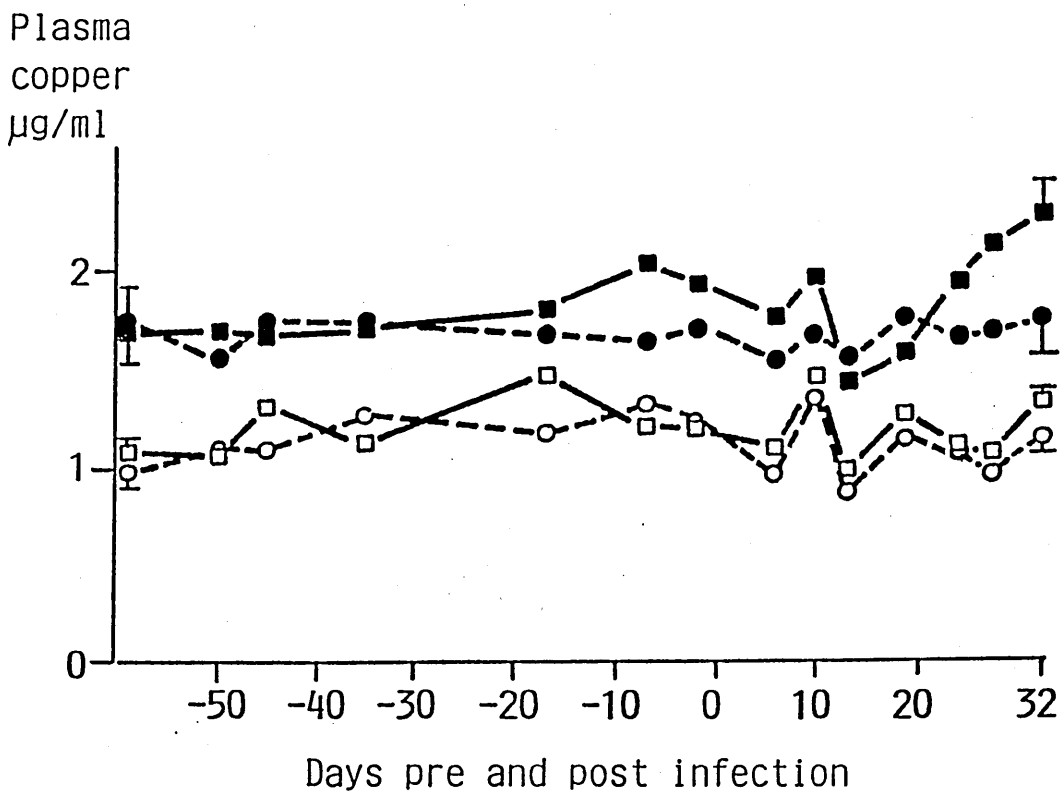

Figure 6b.4 Plasma copper concentration of sheep infected with H. contortus and given drinking water supplements, details of which are shown in Table 6b.1. Group A (■——■) full supplement, Group B (□——□) 10% supplement, Group C (●— —●) molybdate only, Group D (O— —O) no supplement.

Table 6b.3

Plasma copper concentrations (μg/ml) of sheep infected with H. contortus and given water supplements, details of which are provided in Table 6b.1. $\bar{x}$ (± S.E.).

Group	A	B	C	D	Significance:
Supplement	Full Supplement	10% Supplement	Molybdate only	No Supplement	Overall / Between Gps
59 days pre infection	1.68 (0.135)	1.08 (0.041)	1.75 (0.183)	0.96 (0.056)	** A,C > B,D *
2 days pre infection	1.91 (0.125)	1.17 (0.073)	1.67 (0.084)	1.21 (0.052)	** A,C > B,D *
32 days after infection	2.24 (0.186)	1.28 (0.025)	1.712 (0.189)	1.09 (0.057)	** A > B,D *

* $p < 0.05$

** $p < 0.01$

end of which group A values were significantly higher than those of groups B and D (2.24, 1.28, 1.71 and 1.09 ug/ml in groups A, B, C and D respectively).

Liver copper values

The sheep receiving the full supplement (group A) had the lowest mean liver Cu concentration and total liver Cu of all the groups (Table 6b.4). Values from the control group D were significantly higher than those of group A and, for Cu concentration only, group C, while values for the three treatment groups did not differ significantly from each other.

Table 6b.4

The weight, copper (Cu) concentration and content of the livers of lambs infected with H. contortus and given drinking water supplements, details of which are provided in Table 6b.1.

Group	A	B	C	D	Significance:
Supplement	Full Supplement	10% Supplement	Molybdate only	No Supplement	Overall / Between Gps
Liver Wt (g)	587	548	603	551	NS
	(44.8)	(75.2)	(48.8)	(50.7)	NS
Liver Cu (mg/kg FM)	97	147	111	240	**
	(11.8)	(22.5)	(20.7)	(32.5)	D > A,C *
Liver Cu (mg)	58	75	66	132	**
	(9.6)	(7.7)	(11.0)	(24.2)	D > A *

N.S. No significant difference

* $P < 0.05$

** $P < 0.01$

Experiment 6c An investigation of the pathogenicity of *Haemonchus contortus* in parasite-naive and vaccinated Suffolk x Greyface and Finn Dorset sheep aged nine months.

6c.1 Introduction

In Experiment 5, the establishment of *H. contortus* larvae in eight-to-ten month old Suffolk x Greyface sheep was unexpectedly poor compared to that observed previously in younger lambs (Experiments 3a and 4). It was hypothesised that sheep of that age and breed-type might be resistant to the infection.

It was also apparent that although parasite establishment had been normal in these previous experiments, the pathogenic effects on the Suffolk-cross sheep had been much less severe than those recorded in Finn Dorset lambs by Abbott *et al* (1986a) using the same laboratory strain of *H. contortus*. In addition to breed differences, there were differences in nutritional status between their lambs and those of the present series of experiments, and it certainly appears from the results of Abbott (1982), and of Experiments 3a and 4, that nutrient intake influences the consequences of infection. However, there is also known breed variation in susceptibility to haemonchosis and, in particular, Scottish Blackfaces are more resistant than Merino (Abbott, 1982) or Finn Dorset (Abbott *et al*, 1985a) sheep. In addition, it is possible to successfully immunise Scottish Blackface sheep against *H. contortus* from the age of six months (Urquhart, Jarrett and Mulligan, 1962), whereas with Merinos the onset of immunological responsiveness is much later, at some time between 7 and 24 months of

age (Lopez and Urquhart, 1967). The sheep used in the present series of experiments were one-quarter Scottish Blackface, and it was considered that this may have accounted for the observed relative resistance to establishment at eight months, and to the mild pathogenic effects of infection in younger animals.

Therefore, the following experiment was conducted to compare the establishment and pathogenicity of H. contortus in nine-month old Suffolk x Greyface and Finn Dorset sheep, some of which had been previously vaccinated with radio-attenuated H. contortus larvae.

6c.2 Materials and methods

Experimental design

Seven Suffolk x Greyface and nine Finn Dorset sheep aged 7 months were allocated on a random basis to either vaccination (SV and DV, four sheep per group) or challenge control groups (SC and DC, three and five sheep per group respectively).

Sheep of groups SV and DV each received 10,000 irradiated H. contortus larve on two occasions, 28 days apart. Four weeks after the second vaccination all the sheep were given 10,000 normal H. contortus larvae, and after a further 28 days the animals were killed and their worm burdens determined.

Throughout the study determinations of PCV, serum protein concentrations, bodyweights and faecal egg counts were made once or twice weekly.

Experimental animals

The lambs used were castrated males which had been reared parasite-free and from the age of five months were maintained in breed groups on clean straw. They were given 1 kg DM per head per day of a medium protein mix of sugar beet pulp, barley siftings and

soya bean meal, with a mineral supplement, in proportions providing a GE value of 16.5 MJ/kg DM and crude protein content of 120 g CP/kg DM.

Preparation of irradiated larvae

Individual doses of 10,000 H. contortus larvae were prepared by the method described in Section 2 and then exposed to 400 Gy radiation in a ^{60}Co source (Gamma Chamber Mark IVB; Nuclear Engineering Ltd, Reading, Berks, England).

The radio-attenuated larvae were administered to the sheep per os within three hours of preparation.

6c.3 Results

Clinical and bodyweight changes

All the sheep remained clinically normal throughout the study. The Dorset sheep tended to grow a little during the study whereas the Suffolk-cross sheep, which were heavier at the start of the experiment, tended to remain around their original weights (Figure 6c.1).

Parasitological findings

Faecal egg counts

No eggs were detected in the faeces of any sheep during the vaccination phase.

Following the challenge infection, eggs were first detected from the challenge control sheep at 17 or 21 DAI and the mean counts peaked at 10,306 and 22,933 epg at 27 DAI in groups DC and SC respectively.

More variation was observed in egg excretion among the vaccinated sheep. Of group DV, one sheep (DV-105) produced no more than 100 epg whereas egg counts from the other three sheep peaked at

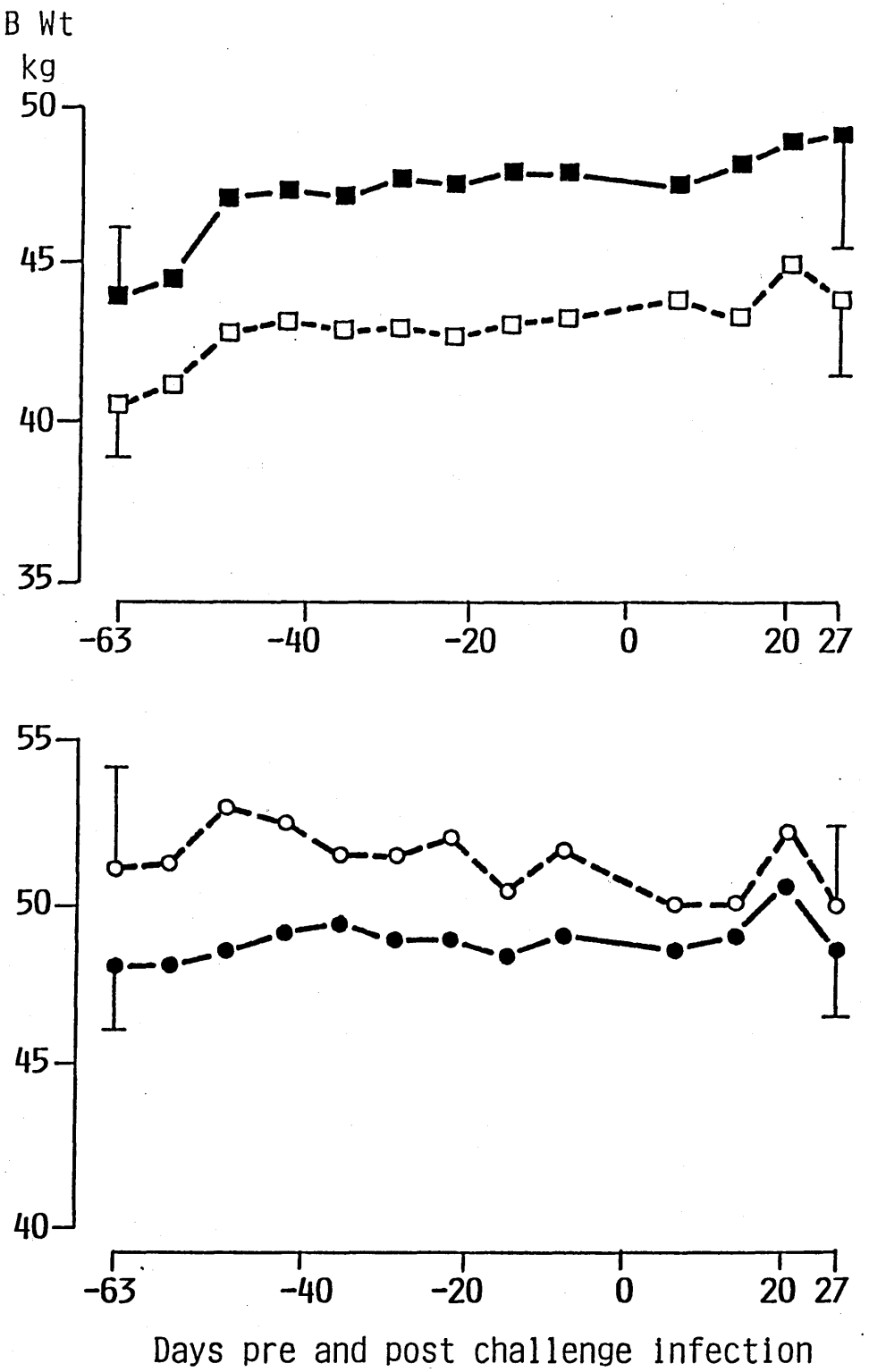

Figure 6c.1 Bodyweight (BWt) of Dorset (■——■) and Suffolk x Greyface (●——●) sheep vaccinated and subsequently challenged with H. contortus larvae, and their respective unvaccinated challenge controls (□———□; O——O).

between 3,200 and 18,200 epg (Table 6c.1). Similarly, in group SV, sheep SV-43 produced no more than 50 epg, while its three companions produced egg counts of 7,900 to 19,600 epg. In SV-43 and SV-94, patency was delayed until 24 DAI.

Group mean egg counts are shown in Figure 6c.2.

Worm burdens

The worm burdens of the individual sheep are shown in Table 6c.1.

Considerably more worms were recovered from sheep of group SC than those of group DC, which reflected the higher faecal egg counts recorded in the former group.

The vast majority of the worms recovered from the challenge control sheep were adults, and the small number of immature forms were all fifth stage larvae.

The vaccinated sheep averaged similar total worm burdens to the controls, although there was considerable individual variation in the numbers of worms recovered and, in a few individuals, a variable but larger proportion of the recovered worms were immature with a considerable number of L_4 stages being identified.

One vaccinated sheep, DV-105, was found to contain no abomasal nematodes at slaughter and had excreted few eggs in its faeces. In SV-94 and SV-43 the effect of vaccination appeared to be an increased proportion of immature worms, associated in the latter sheep with a small worm burden and very low levels of egg excretion. In these two sheep it had been noted that their infections did not become patent until 24 DAI. The worm burdens of the remaining vaccinated sheep did not differ from those of the unvaccinated challenge control sheep.

Table 6c.1

Packed cell volumes (PCV), faecal egg counts (FEC) and worm burdens recovered at slaughter from Finn Dorset and Suffolk cross sheep infected with H. contortus and previously vaccinated (DV and SV) and infected unvaccinated controls (DC and SC).

Group	Sheep No.	27 DAI		Worms recovered 28 DAI				
		PCV(l/l)	FEC(epg)	Adult Male	Adult Female	Immature	Total	% Immature
DV	DV-106	.175	18,200	1,870	2,070	60	4,000	1.5
	DV-105	.295	0	0	0	0	0	-
	DV-101	.295	3,200*	1,920	1,940	0	3,860	0
	DV-104	.25	8,600	1,800	1,350	50	3,200	1.6
DC	DC-108	.225	18,000	1,330	1.470	110	2,910	3.8
	DC-107	.205	17,400	1,960	1,770	10	3,740	< 1
	DC-102	.275	6,800	1,350	1,410	10	2,770	< 1
	DC-103	.275	9,200	1,510	1,070	170	2,750	6.2
	DC-109	.195	1,300	760	810	0	1,570	0
SV	SV-89	.25	19,600*	2,190	2,410	70	4,670	1.5
	SV-2	.25	9,200*	2,440	2,380	70	4,890	1.4
	SV-94	.255	7,900	1,340	1,330	700	3,370	20.8
	SV-43	.305	50	310	120	620	1,050	59.0
SC	SC-95	.225	22,400	2,200	2,550	40	4,790	< 1
	SC-97	.23	24,800	2,560	2,290	60	4,910	1.2
	SC-92	.25	21,600	2,000	1,890	10	3,900	< 1

* Measured 24 DAI

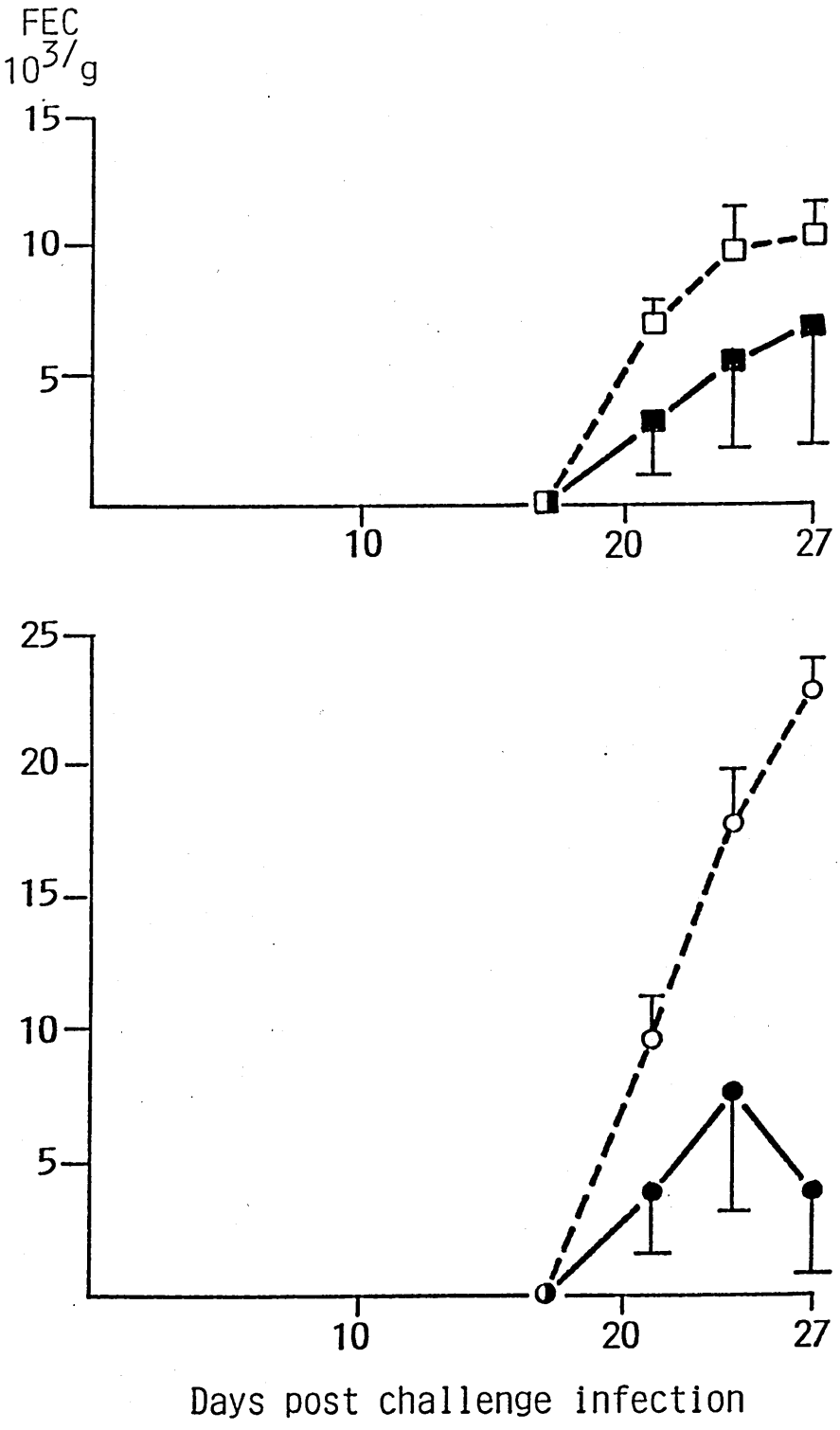

Figure 6c.2 Faecal egg count (FEC) of Dorset (■——■) and Suffolk x Greyface (●——●) sheep vaccinated and subsequently challenged with H. contortus larvae, and their respective unvaccinated challenged controls (□——□; O——O).

Haematocrits

A slight fall in PCV was evident in all the groups during the vaccination phase. From around 10 days after the challenge infection a more dramatic fall in PCV occured reaching lower values among the control sheep (0.224 and 0.230 l/l in groups DC and SC) than in those previously vaccinated (0.241 and 0.261, groups DV and SV) (Figure 6c.3).

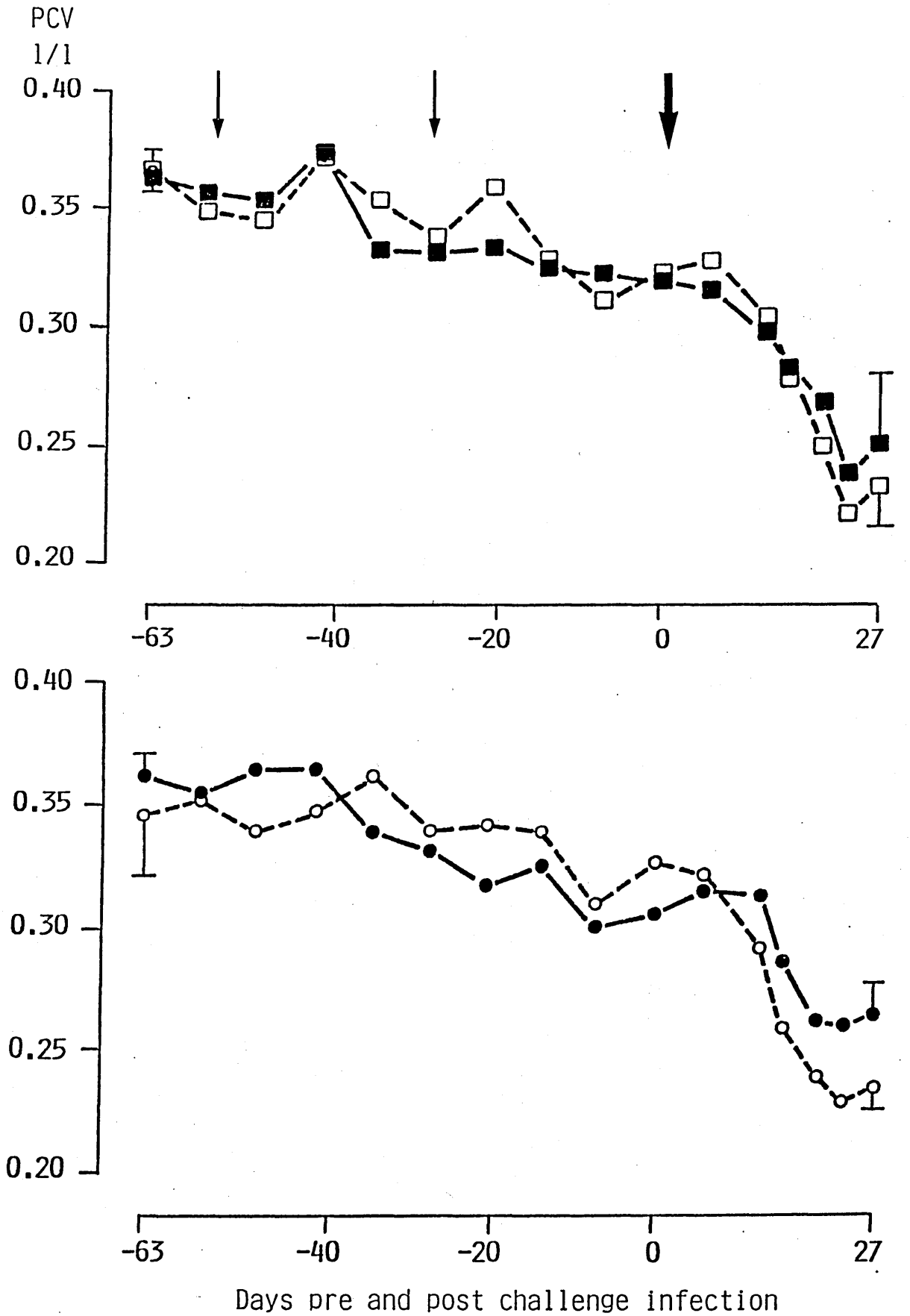

Figure 6c.3 Packed cell volume (PCV) of Dorset (■——■) and Suffolk x Greyface (●——●) sheep vaccinated and subsequently challenged with H. contortus, and their respective unvaccinated challenged controls (□——□; O——O).

↓ Vaccination ↓ Challenge Infection

6.4 Discussion

These experiments were conducted as an investigation into possible reasons for the unexpectedly low parasite establishment and pathogenicity observed in Experiment 5.

The hypotheses examined in the three experiments were firstly, that the larval strain used had become less pathogenic; secondly, that prophylactic medication against copper toxicity had an anthelmintic effect; and thirdly, that sheep of the breed-type used develop resistance to the infection by the age of nine months.

In the first experiment, Scottish Blackface lambs were infected with either the suspect Glasgow strain of *H. contortus*, or a Babraham strain of known pathogenicity. Scottish Blackface sheep have been previously shown to be relatively resistant to the pathogenic effects of haemonchosis, compared to Merino (Abbott, 1982) and Finn Dorset lambs (Abbott *et al*, 1985a & b). In this study, both strains of larvae established patent infections and caused anaemia and, in fact, larvae of the suspect Glasgow strain produced a greater reduction in PCV, and higher faecal egg counts and worm recovery than did the Babraham larvae. Only one sheep was infected with each strain, and thus no concrete conclusions can be drawn from this small study. However, the results do tend to confirm the pathogenicity of the Glasgow strain of *H. contortus*.

The second experiment investigated the possible anthelmintic effect of ammonium molybdate and sodium sulphate given over three months.

All three treatments resulted in lowered liver Cu levels, the effect being greatest with the full original supplement, followed by the molybdate component only, while the administration of 10% of the

original supplement had least effect. Although clinical copper toxicosis did not develop within the span of this study, liver Cu concentrations in four of the five untreated group D sheep, but none of the treated animals, were above the level regarded as dangerously high by Suttle (1974), 750 mg/kg DM. From this one can conclude that the supplements were effective in reducing the absorption and, or, the storage of Cu, and in maintaining liver Cu stores at a safe level.

Plasma Cu concentrations proved harder to interpret, the main effects observed being that values in groups A and C were consistently higher than those of the other groups, and in group A an increase was apparent during the latter stages of the study.

Suttle (1977) observed that plasma Cu levels were not affected by treatment with Mo and S unless animals were approaching a haemolytic crisis. However, the rise in plasma Cu observed in group A probably reflected a systemic effect of absorbed thiomolybdates, rather than the onset of a haemolytic crisis, given that these sheep had the lowest liver Cu values (Mason, 1981). Robinson, Devlin, Wittenberg and Stanger (1987) also found plasma Cu to be a relatively insensitive index of Cu status. It would have been interesting to have evaluated a more sensitive parameter, such as plasma ceruloplasmin oxidase (Robinson et al, 1987) or benzylamine oxidase (Mason, 1981), or to have measured liver enzymes, Sharman (1983) having indicated that blood amino-aspartate transaminase (AAT) is elevated when liver Cu exceeds 750 mg/kg DM.

The main aim of this experiment was, however, to assess the effect of the thiomolybdate supplement on the establishment and pathogenicity of H. contortus larvae, since previously such

administration had been coincident with poor parasite 'takes' in Experiment 5. In this experiment, the treatments caused no lessening of the pathogenic effects of haemonchosis on PCV or serum protein concentration, or on faecal egg counts or worm burdens at slaughter. In fact, the animals receiving the maximal supplement were, on average, most affected by the infection, in terms of drop in PCV and serum protein concentration, and they exhibited the shortest prepatent period and greatest parasite establishment. This showed that, if anything, the supplement might have enhanced the establishment of the parasites. The only sheep in the experiment (or in any experiment recorded in this thesis) to show clinical signs of acute haemonchosis was a small lamb in group A which developed clinically apparent anaemia and submandibular oedema. Although individual larval doses were calculated on a bodyweight basis, and this lamb received the fewest worms, it seems likely that its small size was a factor in its poor ability to cope with the infection.

Suttle, Knox, Jackson and Coop (unpublished presentation to AVT&RW meeting, Scarborough, 1988) observed low parasite egg counts in the faeces of sheep affected by Mo-induced Cu deficiency, and this prompted them to study parasite development in sheep with induced Cu deficiency. Two groups of lambs fed a cereal-based diet were given 500 H. contortus larvae/d, 5 d/week for six weeks, and one group received a dietary supplement of 5 mg Mo/kg DM. Faecal egg counts, from 33 DAI, and worm recovery after 56 days, were much reduced by the Mo, although both groups became similarly anaemic. Thus, there seemed to be increased pathogenicity of the fewer established worms.

These results seem at variance with those recorded in the present Experiment 6b, but it should be remembered that Suttle and

his colleagues were dealing with sheep rendered Cu deficient by the Mo supplement. In contrast, the sheep in Experiment 6b were believed to be of adequate or high Cu status (although more sensitive assessment of Cu status may have suggested otherwise), and the Mo supplement was administered here to prevent copper toxicosis rather than to induce a deficiency state.

Experiment 6c proved inconclusive. The hypotheses under examination were that the Suffolk x Greyface challenge control sheep might prove more resistant to parasite establishment and less susceptible to the pathogenic effects of haemonchosis than the equivalent Finn Dorset animals, and that the Suffolk x Greyface sheep would be able to respond effectively to vaccination against H. contortus. However, neither of these hypotheses were supported by the results.

Comparing the challenge control groups of the two breed-types, it is apparent that both proved susceptible to infection, and parasite establishment and egg excretion were in fact slightly greater among the Suffolk-cross sheep compared to the Dorsets. Worm fecundity, expressed as eggs per g faeces, per worm recovered, was also slightly greater among the Suffolk-cross sheep. The effects of the infections on PCV were comparable in the two groups.

In this experiment, 10,000 larvae were administered to each sheep, and this therefore represented a slightly lower challenge, on a bodyweight basis, to the heavier Suffolk x Greyface. In all the sheep, the larval challenge was lower than that used in the previous experiments, ranging from 270 (given to sheep DC-108) to 170 larvae/kg BWt given to the largest sheep, SC-92. Despite the infective challenges being lower, the effects on PCVs were similar to

those seen in the previous experiments. This may have reflected a difference in nutritional status, as the mean nutrient intake in this experiment was less than in any of the previous studies.

Hamed (1985) compared the pathogenesis of haemonchosis in 6 month old Finn Dorset and 4 month old Suffolk x Greyface lambs given 350 larvae/kg BWt and consuming 1 to 1.5 kg Super Star cubes/d. Anaemia was slightly more severe in the Finn Dorset group, with the mean PCV reaching 0.16 l/l compared to 0.19 among the Suffolk-cross sheep. Additionally, faecal egg counts were slightly higher in the Finn Dorset sheep, although parasite establishment was slightly greater among the Suffolks. Pathophysiological studies revealed that gastrointestinal blood losses were larger among the Finn Dorsets, although circulating red cell and blood volumes were lower in the Suffolk-cross lambs. The average blood loss per parasite per day was 0.04 ml and 0.02 ml in the Finn Dorset and Suffolk-cross sheep respectively. These results led her to conclude that there was a greater degree of resistance in the Suffolk x Greyface compared to the Finn Dorset sheep, and that this was manifested as a greater capacity to cope with the pathogenic effects of the parasites rather than to limit their establishment.

The results of the present experiment do not contradict this view, but in themselves present little evidence of a marked breed difference in either the establishment or pathogenicity of the parasite.

Considering the effects of vaccination, it is apparent that neither breed type proved particularly responsive. One Dorset sheep, DV-105, was rendered solidly immune, and the challenge infection failed to establish in this sheep. The other DV sheep

proved to be fully susceptible to the challenge infection, although the effect on haematocrits was slightly less than in their challenge control counterparts.

Among the Suffolk x Greyface vaccinated lambs, there was some evidence of an immune response in two sheep, manifested as an increase in the proportion of immature forms found at slaughter at 28 DAI. This was accompanied by a delay in patency in these two animals.

This response to vaccination is, however, minor compared to the strong resistance to challenge infection observed in vaccinated eight-month old Scottish Blackface sheep by Urquhart et al (1962), and Abbott (1982). Certainly, this present vaccination experiment in no way suggests that the poor parasite establishment recorded in Experiment 5 reflected an age-related resistance to H. contortus in Suffolk x Greyface sheep.

Thus, all three experiments in this Section support the null, rather than the experimental, hypotheses, and the explanation for the low pathogenicity of the infections in the previous Section remains elusive.

SECTION 7

Pathophysiological effects associated with larval challenge of immune animals.

Section 7

Pathophysiological effects associated with larval challenge of immune animals.

7.1 Introduction

Helminthiasis is normally regarded as a problem of young stock and the results of many studies, including those presented in this thesis, have demonstrated important pathophysiological changes and production losses in growing animals. However it is apparent that older animals, which are judged to be immune on the basis of low parasite burdens or faecal egg counts, may nevertheless show production losses such as reduced wool production (Barger et al, 1973; Barger and Southcott, 1975) when exposed to larval challenge.

A pathophysiological basis for such loss of production was hinted at when Anderson (1973) reported elevated plasma pepsinogen values in adult (and theoretically immune) sheep exposed to a field infection of predominately O. circumcincta, despite low or negative faecal egg counts from the sheep.

Yakoob et al (1983) described an experiment in which mature ewes were challenged for seven and later nine days with 7,000 O. circumcincta larvae daily. Plasma pepsinogen levels were doubled in the challenged sheep. At the second challenge period, when radioisotopic studies were conducted, it was found that challenged sheep had greatly elevated losses of plasma into the GI tract, and major increases in the rate of albumin catabolism.

In the present study ewes with a similar grazing history to those used by Yakoob et al (1983) were exposed to challenge with

O. circumcincta larvae for a prolonged period (121 days) to examine the duration of the pathophysiological changes associated with larval challenge.

Experiment 7 Pathophysiological changes in adult sheep challenged with Ostertagia circumcincta.

7.2 Materials and methods

7.2.1 Experimental design

Eight mature non-pregnant Blackface ewes which had previously grazed pasture contaminated with a mixed trichostrongyle infection were allocated to three groups. Groups A and B each contained three sheep, and Group C two sheep. The hot dry summer of 1984 produced low pasture larvae counts, so the ewes were housed in mid-August and each sheep of Groups A and B received a daily oral dose of 1,000 Ostertagia circumcincta larvae to simulate a moderate field challenge. Group C sheep were treated with fenbendazole and received no larvae.

Five weeks after housing, larval dosing was stopped and all the ewes were treated with fenbendazole. Nineteen days later, larval challenge of Group A was recommenced, each sheep receiving 7,000 O. circumcincta larvae daily for 121 days.

Throughout the study each sheep was offered 1500 g fresh matter per day of Super Star Cubes, the complete pelleted ruminant feed described in Section 3.

The sheep were moved to metabolism stalls on three occasions for radioisotopic studies of gastrointestinal plasma loss and albumin catabolism using $^{51}CrCl_3$ and ^{125}I-albumin. These were from days 1 to 15 of challenge, and from days 36 to 48 and days 108 to 121 of challenge. The radioisotopic techniques used essentially followed the methods described in Section 2, but with the exception that as the sheep were female, faeces was collected in nylon mesh

bags to allow urine to pass through. Radioactive determinations were made on samples of faeces taken from both the rectum and the mesh bag to allow estimation of, and correction for, the amount of urine contaminating the faeces in the bag.

The sheep were killed on day 121 and their gastrointestinal tracts examined for nematodes.

Faecal egg counts, haematocrits, body weights, plasma pepsinogens and serum protein concentrations were measured once or twice weekly throughout the experiment by the methods described in Section 2.

7.2.2 Statistical analysis

Differences between Group A, and Groups B and C combined, were assessed using the Mann-Whitney test and analysis of variance as appropriate.

7.3 Results

7.3.1 Clinical observations

One sheep from Group A (A3) developed diarrhoea after 24 days of challenge. This persisted for three days after which its faecal consistency returned to normal. All other sheep produced pelleted faeces throughout the experiment.

Sheep B1 showed a variable appetite throughout the experiment and periods of inappetance were associated with reduced output of urine and faeces. During the second isotope study, A2 also showed a depressed appetite. During the third study, all the sheep except A3 ate slightly less than they had previously eaten. No other abnormal clinical signs were detected.

7.3.2 Bodyweight changes

All the sheep but one gained a little weight during the

experiment, the exception being B1 whose bodyweight fluctuated around its original weight. The mean gain over the course of the study was 5 kg in each group (Figure 7.1).

7.3.3 Parasitological findings

Faecal egg counts

Prior to anthelmintic treatment, small numbers of eggs (< 500 epg) were found in the faeces of all the sheep except A3. During the challenge period eggs were observed on one occasion each in faeces from A1 and A2 (50 and 200 epg, 18 and 101 days post-challenge respectively). No eggs were observed from A3 or from sheep of Groups B and C.

Post-mortem worm burdens

Only one sheep contained gastrointestinal parasites at post-mortem examination. A2 had a burden of 900 O. circumcincta fourth stage larvae. This represents one tenth of one per cent of the larvae administered to the sheep during the experiment.

7.3.4 Haematology and blood biochemistry

Haematocrits

Larval challenge had no effect on haematocrit values which remained within normal range in all sheep throughout the study.

Serum proteins

Averaged over the challenge period, slight hypoalbuminaemia and hypoproteinaemia were evident in the challenged ewes compared to the controls (35 and 73, and 38 and 77 g/l in Group A and Groups B and C combined, respectively).

Plasma pepsinogen concentrations

During the preliminary stimulation challenge, group mean pepsinogen values rose to 1.5 iu in Groups A and B, while Group C

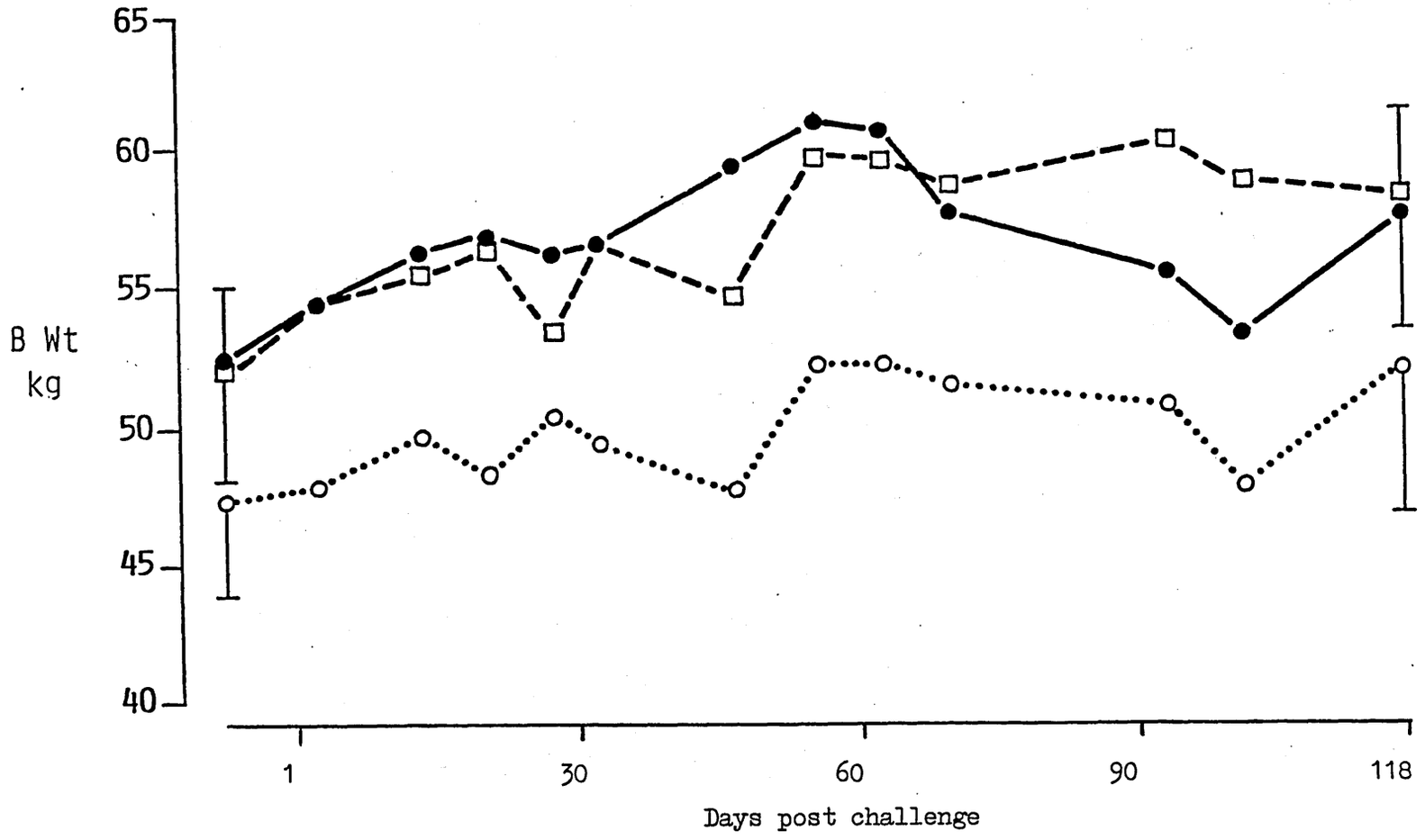

Figure 7.1 Bodyweight (BWt) of mature sheep challenged with O. circumcincta larvae (Group A, ●——●) and previously challenged (Group B, O·····O) and unchallenged (Group C, □--□) controls.

remained around 0.6 iu. Following anthelmintic treatment at the cessation of challenge, mean values fell quickly to 0.6 iu in all groups (Figure 7.2).

After the experimental challenge was commenced, the mean plasma pepsinogen value of Group A rose rapidly to over 1.0 iu, and remained at or above 1.0 iu throughout the experiment. Mean values for Groups B and C remained around 0.5 iu for the remainder of the experiment.

Within Group A there was considerable individual variation in plasma pepsinogen response. A1 showed the lowest plasma pepsinogen of any sheep in the experiment, 0.1 iu, and this did not increase in response to larval challenge. In contrast A2 and A3 showed increases of about 1.0 iu within 10 days of commencing challenge, and showed peak pepsinogen values of 2.3 and 3.0 iu respectively at day 74. By the end of the experiment, values in these two sheep had both fallen to around 1.6 iu.

7.3.5 Radioisotopic studies

$^{51}CrCl_3$ Plasma losses into the gut

Before challenge the values were similar in all groups, around 80 ml per sheep per day (Figure 7.3). Nine days after challenge two sheep of Group A showed an increase in plasma loss. A2 showed two peaks of plasma loss - 140 and 160 ml/day at days 12 and 45 of challenge. A3 showed the biggest increase in response to challenge, and its plasma losses remained the highest throughout the experiment, reaching 200 ml at day 45. The plasma loss of sheep A1 remained at control levels throughout the study. During the third isotope study, plasma losses in all sheep were slightly lower than previously observed.

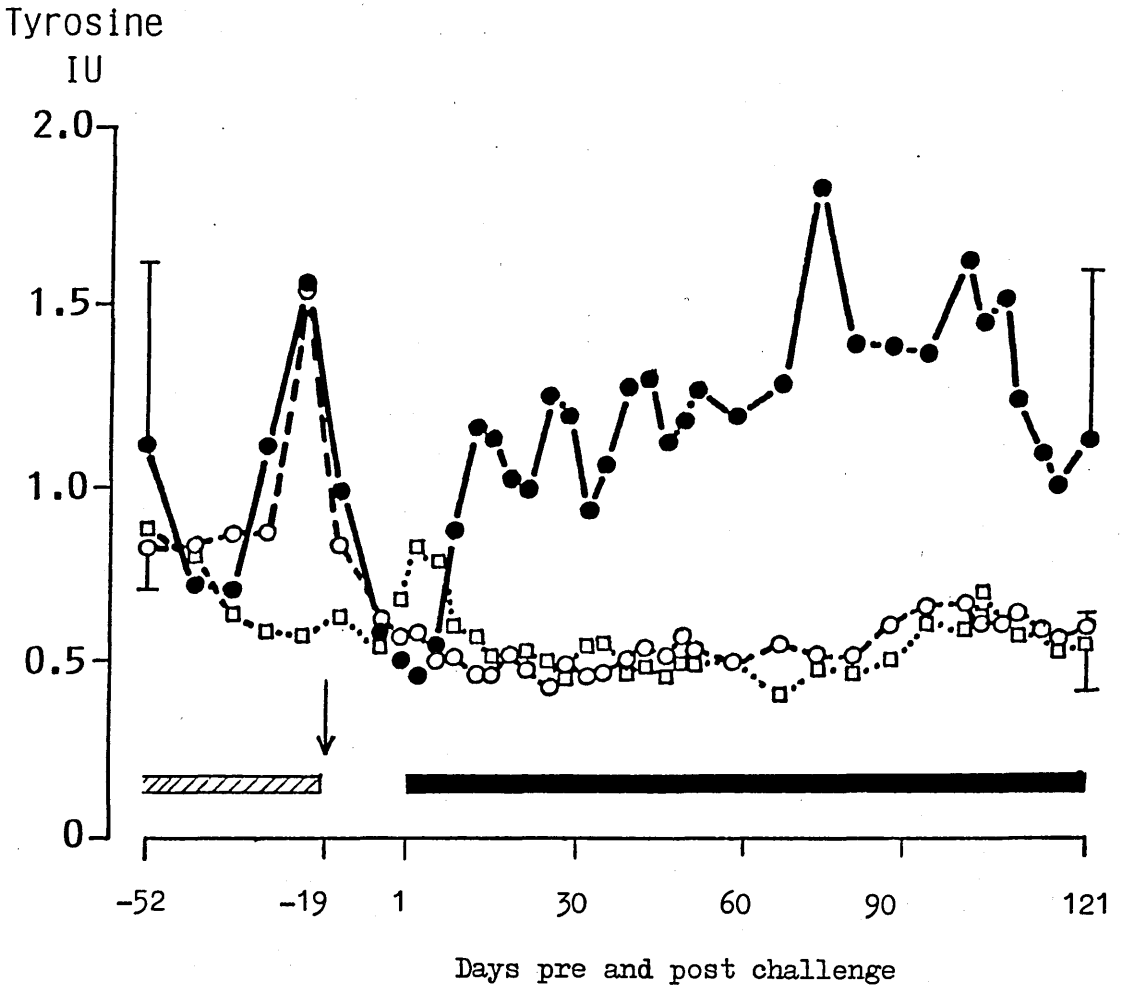

Figure 7.2 Plasma pepsinogen activity (IU tyrosine) of mature sheep challenged with O. circumcincta larvae (Group A, ●——●) and previously challenged (Group B, O--O) and unchallenged (Group C, □·····□) controls

Stimulation infection of Groups A and B
Anthelmintic treatment of all sheep
Experimental challenge of Group A.

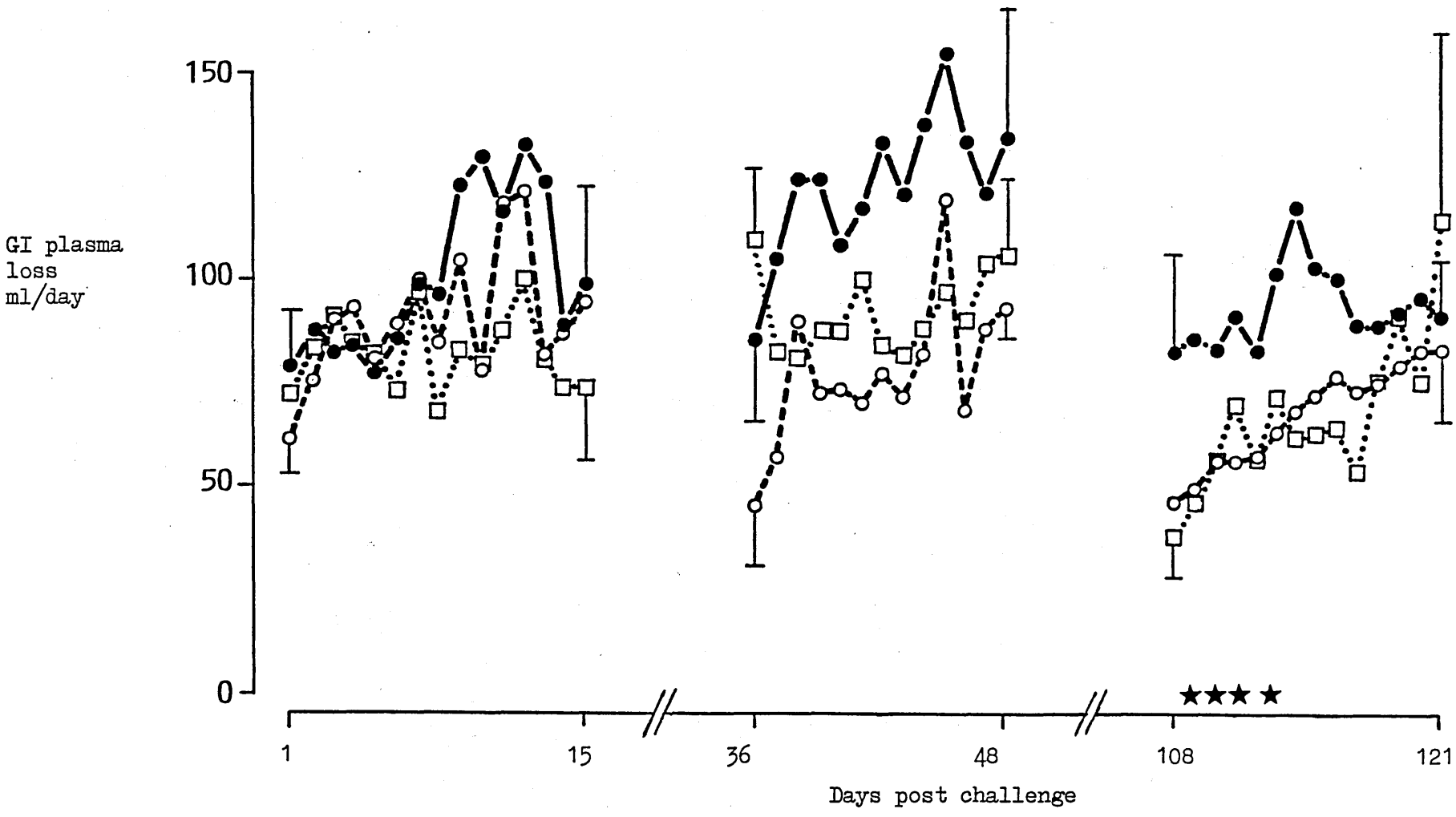

Figure 7.3 Gastrointestinal (GI) loss of plasma in mature sheep challenged with O. circumcincta (Group A, ●——●) and previously challenged (Group B, O- - -O) and unchallenged (Group C, □·····□) controls.

Significance: ★ A > B + C $p < 0.05$

Because of the individual variation, the differences between the groups were only significant at days 109, 110, 111 and 113 of challenge when Group A losses were significantly higher than the losses of Groups B and C combined.

Albumin half-life

The half-life of albumin in plasma tended to be lower in the challenged sheep, and this difference was statistically significant at the third study period (Table 7.1).

Fractional rate of albumin catabolism

A2 and A3 tended to have higher fractional catabolic rates than the other sheep throughout the four month study (Figure 7.4). Group A rates were significantly higher than those of Groups B and C on occasional days, and Group A sheep had significantly higher mean catabolic rates during the third study than controls.

Table 7.1

The half life ($T\frac{1}{2}$) of albumin in plasma of adult sheep challenged with O. circumcincta larvae (A) and unchallenged controls (B,C) measured at various times after first challenge.

Sheep	Albumin $T\frac{1}{2}$ (h)		
	0 - 15 days	36 - 48 days	108 - 121 days
A1	460	400	394
A2	518	297	410
A3	475	407	396
B1	576	479	512
B2	588	591	464
B3	481	386	419
C1	529	453	551
C2	593	484	421
Significance	N.S.	N.S.	*

* A < B + C; P = 0.05

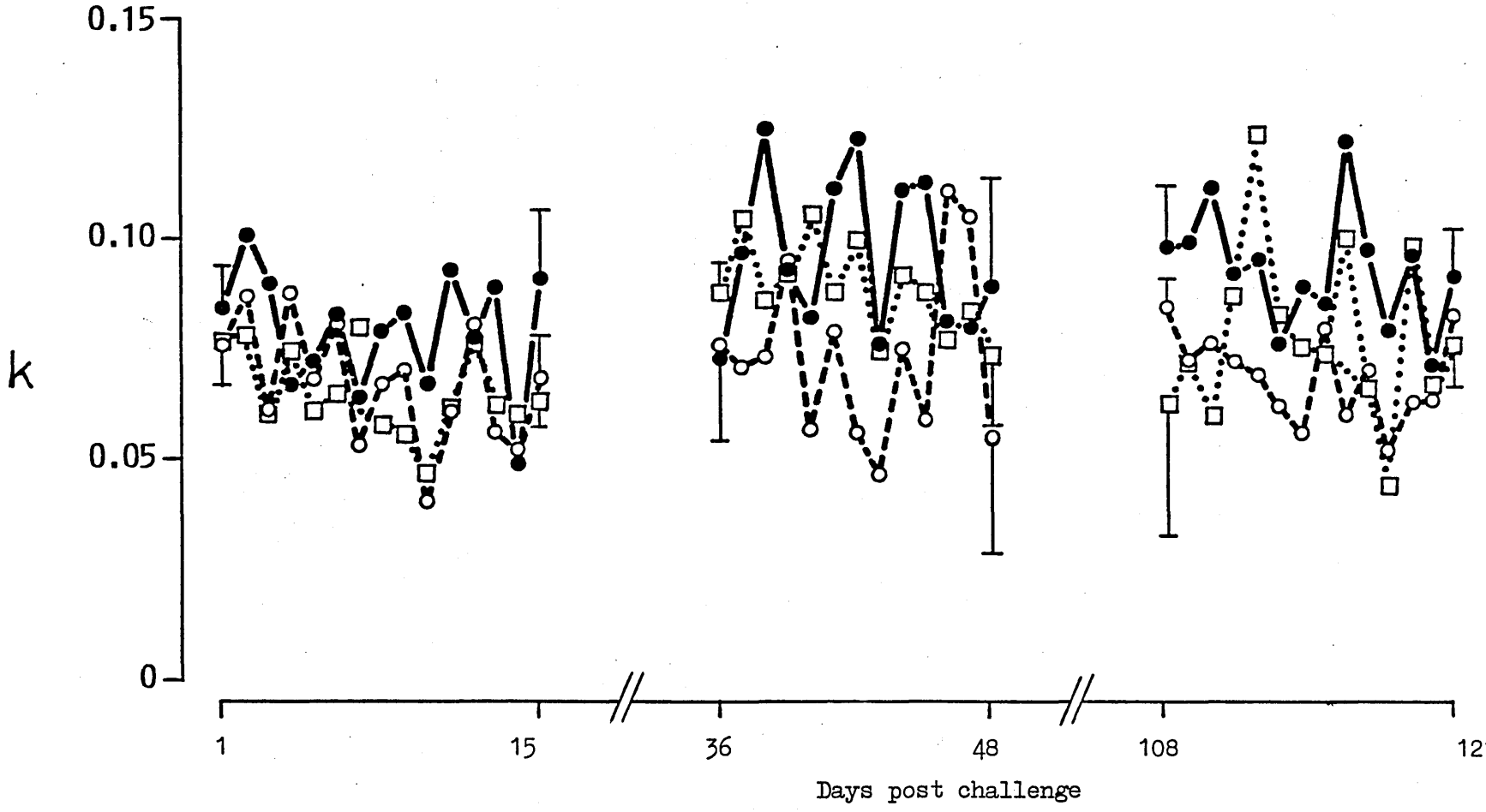

Figure 7.4 Fractional rate of albumin catabolism (k) in mature ewes challenged with O. circumcincta (Group A, ●——●) and previously challenged (Group B, O——O) and unchallenged (Group C, □·····□) controls.

7.4 Discussion

This experiment, using ewes with a similar grazing history to those used previously by Yakoob et al (1983), but in which larval challenge was continued for a much longer period of 121 days, demonstrated that the pathophysiological changes associated with larval challenge could continue for several months.

Confirmation that such effects can persist for a prolonged period is of considerable importance. If the changes occured only transiently at the commencement of exposure to larval challenge it could be argued that the overall metabolic cost to the sheep would be small. However, the prolonged gastrointestinal disturbance demonstrated here can be assumed to represent a considerable drain on the ewe's metabolic resources, and this may reasonably be proposed as the underlying cause of reduced production reported in challenged immune ewes by previous workers (Anderson, 1973; Barger and Southcott, 1975).

The sheep of Group B, which were previously challenged, treated with anthelmintic, and then maintained unchallenged during the experimental period, did not show evidence of any pathophysiological disturbance. Hence, Groups B and C (entirely unchallenged) were essentially duplicate control groups, and data from the two groups were therefore combined for comparison with the challenged Group A.

It has been previously shown that, in parasite-naive sheep infected with H. contortus, removal of infection with fenbendazole was followed by a return of GI plasma losses to control values within four days, paralleling the disappearance of strongyle eggs from faeces (Harrison, Hills, Holmes and Stevenson, 1985). These findings, and the results from the present control groups, tend to

suggest that previous parasitic challenge, followed by successful removal of infection with an effective anthelmintic, does not result in continued pathophysiological disturbance after exposure to the parasites has ceased.

Among both the challenged and control groups in the present study, plasma albumin half-lives were longer, and catabolic rates were lower, than in the comparable infected and control groups of growing lambs in Experiment 4. This is perhaps a reflection of a lower rate of protein metabolism in these non-productive mature animals.

In the experiment of Yakoob et al (1983), the challenged sheep excreted strongyle eggs and accrued worm burdens of 302 to 3,202 mainly Ostertagia spp., with 25 % being adult. It could thus be argued that their ewes, although relatively resistant compared to parasite-naive lambs, were only partially immune to the parasite species administered. In contrast, in the present experiment only one sheep, A2, retained worms at slaughter, and these were 900 immature stages. This suggests that the present sheep had a higher degree of immunity to O. circumcincta than those of the previous workers. Although the level of immunity, as evidenced by worm burdens at slaughter, may have been enhanced in the present experiment by continued exposure to larvae throughout the study, it is apparent from comparison of faecal consistency and nematode egg counts early in the study that the present sheep were more solidly resistant than those of Yakoob et al (1983).

This difference in immune status is important because it counters the possible argument that the pathophysiological changes in the sheep of Yakoob et al (1983) occured because the sheep were

only partially resistant to the parasites. In the present experiment, immunity as measured by conventional parasitological parameters was much more solid, and differences in albumin catabolism and GI plasma losses, though slightly smaller, were still apparent.

It is of considerable interest that the pathophysiological response recorded in this experiment varied between individual sheep with two ewes showing four-fold increases in plasma pepsinogen levels and doubled losses of plasma proteins, whilst another showed no response to larval challenge.

Although not alluded to in their text, it is apparent from examination of standard errors on the graphs of Yakoob et al (1983) that within the challenged group A they too had at least one sheep which showed little response to challenge in terms of the rate of albumin catabolism and gastrointestinal plasma loss. It would appear, however, that all three of their challenged sheep showed an increase in plasma pepsinogen value, whereas in the present experiment, sheep A1 showed no response to challenge in any of the parameters measured.

One can only speculate as to why sheep A1 showed this complete lack of response. It may be that this sheep represented the totally resistant sheep, and that in fact larval challenge has no effect on such animals. That was the impression gained by Wagland, Steel and Dineen (1982) who rendered young sheep highly resistant to T. colubriformis by administration of three doses of 60,000 irradiated larvae at four-weekly intervals. They found that whilst wool production was impaired in these animals during the vaccination period, later larval challenge was not accompanied by reduction in liveweight gain or wool growth.

Against this argument is the observation that the one sheep in the present experiment from which no eggs were ever recovered in faeces, A3, was the sheep which demonstrated the greatest pathophysiological response to challenge.

It is apparent that this is an area which merits further investigation, but to put the issue of complete versus relative resistance into context it should be pointed out that the sheep used in the present experiment and that of Yakoob et al (1983) were randomly selected Blackface ewes acquired through commercial markets in the West of Scotland. They are therefore likely to be representative of the adult sheep population which have conventionally been considered to be resistant to the effects of parasite challenge. Despite limited parasitological evidence of infection, the majority of the experimental sample nonetheless showed pathophysiological changes of a magnitude previously associated with production losses in naive sheep subjected to trichostrongylid infection (Holmes and Maclean, 1971; Symons et al, 1981) and, further, these losses persisted for several months under continued exposure to infective larvae.

Yakoob et al (1983) and Holmes and Armour (1985) discussed the potential mechanism underlying these pathophysiological changes. They considered that the macromolecular leak across the abomasal wall was probably not a response to the physical effects of maturing larvae and adult parasites as is the case in primary infections. Rather, the more prompt response in resistant animals may be the result of an immune-mediated hypersensitivity reaction of the abomasal mucosa to ingested larvae, similar to that previously described by Barth, Jarrett and Urquhart (1966) in rats undergoing a

self-cure reaction to the nematode Nippostrongylus brasiliensis. A summary of this mechanism is shown in Figure 7.5.

The measurement of blood pepsinogen is well established as an aid to the diagnosis of clinical ostertagiasis in cattle (Armour, Bairden, Duncan, Jennings and Parkins, 1979), although the interpretation of moderately raised levels in clinically healthy, previously exposed, second-year grazing animals has caused some debate (Armour et al, 1979; Entrocasso, McKellar, Parkins, Bairden, Armour and Kloosterman, 1986a). As in the sheep in the present experiment, these values represent a response to larval challenge, rather than the development of large numbers of larvae to the adult stage, and this has led some workers to dismiss these increases as of no practical relevance. However, as the present experiment shows, in sheep at least, the increase in plasma pepsinogen may be accompanied by substantial gastrointestinal plasma losses and alterations in blood protein metabolism of a magnitude previously associated with production losses in naive sheep. Although not necessarily accompanied by overt clinical disease, an increase in plasma pepsinogen in older animals should be considered to be at least a warning sign that the animals are undergoing pathophysiological changes as a result of exposure to larval challenge, that these effects last throughout the period of exposure, and that impaired production may well be a consequence.

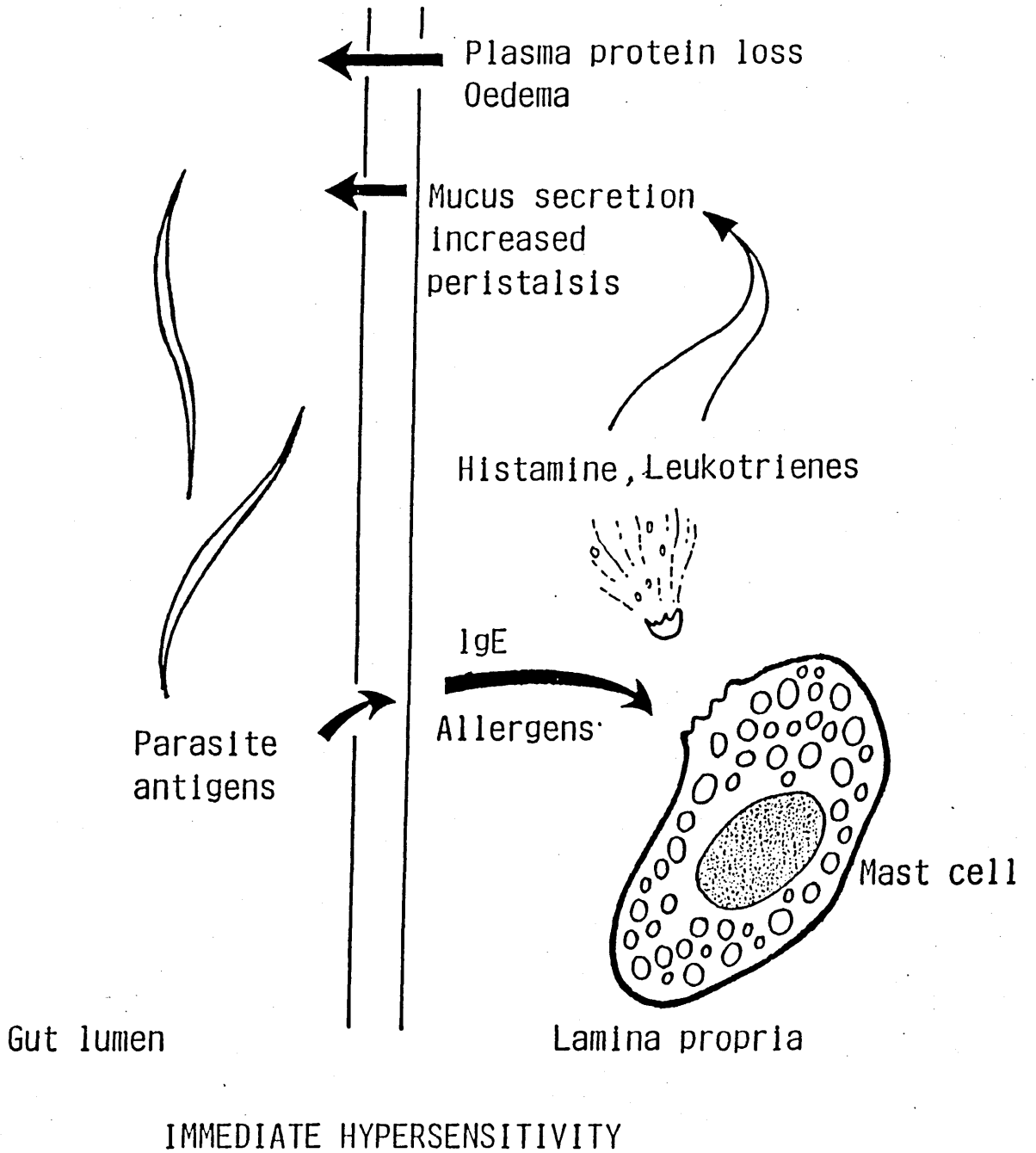

Figure 7.5 Proposed mechanism for an immune-mediated hypersensitivity reaction to ingested larvae in the abomasal mucosa of immune animals (based on Barth et al, 1966).

SECTION 8

General discussion and conclusions.

Section 8

General discussion and conclusions.

The work presented in this thesis provides evidence of the deleterious effects of trichostrongylidosis on animal production and insights into the mechanisms underlying these effects. Further, it highlights several areas which would merit further investigation in the quest for understanding of the pathophysiological and metabolic consequences of parasitism.

The experiments involving H. contortus infections were characterised by relatively mild pathogenicity resulting, in most cases, in subclinical disease, or what may be described as mild chronic haemonchosis, in the classification of this parasitism by Allonby (1973) and Allonby and Urquhart (1975). Infection caused a moderate macrocytic, normochromic anaemia with few other clinical signs, and with appetite and growth rates being indistinguishable from those of uninfected sheep.

Experiments 3a and 3b were conducted to measure the effects of infection with H. contortus on the intake and utilisation of a complete pelleted diet by growing lambs. The diet proved highly palatable, feed intakes were high and not reduced by infection, and there were no significant effects detected on growth or body composition following either single acute, or repeated trickle, infections with the parasite. There was some reduction in ration digestibility in the sheep given the single infection, six weeks after infection, and nitrogen retention was reduced by both patterns of infection at that time.

The hypothesis that the mild nature of the disease in Section 3 might be the result of the very high feeding levels was examined in Experiment 4, by restricting feed intakes to one of two lower levels. Again, the effects of the parasites were quite mild, but the experimental hypothesis was supported by the observation that the lower feeding level was associated with more profound effects of infection on body composition, and in some parameters at least, there was statistically significant evidence of an interaction between parasitism and nutrition. It is evidence of such an interaction that has been sought by previous workers (eg. Abbott et al, 1986a; 1986b), but unfortunately they did not subject their data to appropriate statistical analysis, instead being content to compare group differences with a series of t-tests, a technique which cannot be recommended on grounds of either sensitivity or validity.

Sections 3 and 4, taken together, provide good evidence that the effects of haemonchosis on health and production can be largely mitigated by generous nutrient intake. With sequential reductions in feed intake, production effects became apparent and more pronounced, and this is consistent with the field observation from Kenya that the syndrome of chronic haemonchosis is associated with poor nutrition in the dry season (Allonby and Urquhart, 1975). Abbott (1982) demonstrated the effect of dietary protein concentration on the susceptibility of sheep to haemonchosis. In her studies, sheep given the lower protein diet suffered more profound effects of infection, although parasite establishment was similar in the two dietary groups. Anorexia was a feature of the disease only in the low protein infected sheep. A significant feature, largely undiscussed by Abbott, was that the inappetance

apparent among the low protein infected sheep resulted in their intake of all nutrients being considerably reduced, and the effects she was attributing to reduced protein intake may have been the result of a deficiency of other nutrients, for example, dietary energy.

Measurement of parasitic effects on body composition in the present studies was hampered by the relatively mild nature of any such changes which thus required particularly sensitive methods of investigation for their detection. Additionally, a series of technical difficulties reduced the usefulness of some of the techniques in each experiment. This precluded the intended assessment and comparison of indicator joint dissection, versus chemical or neutron activation analysis of whole body homogenates, as suitable and convenient analytical techniques for the detection of production effects of trichostrongylidosis. Despite this limitation, whole body analysis in Experiment 4 did demonstrate for the first time that haemonchosis can affect the composition of body gain and, in particular, that infected sheep gained less protein, fat and energy per unit gain in body tissue than uninfected, pair-fed controls. The effect of feeding level and infection appeared to be not merely additive as, at least in the case of the retention of dietary energy over 42 days, a significant interactive effect was noted with the infected sheep given the smaller ration retaining disproportionately little dietary energy.

The endocrine studies conducted in Experiment 3a produced a few interesting findings, but given the mild effects of the infection on other parameters, the only conclusion that can be supported with confidence is that this is an area which should prove worth studying

in sheep more profoundly affected by haemonchosis.

Experiment 4 confirmed the value of radioisotopic tracer techniques in assessing factors influencing the pathogenicity of parasitic infections. Such techniques, although not new, provide valuable direct evidence 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 only an indirect measurement of pathogenicity.

The study of the partitioning of dietary energy in haemonchosis (Section 5) was unfortunately limited by the low establishment and pathogenicity of the infections. However, there was evidence of altered rumen function, in the form of increased methane production in parasitised sheep. This again is an area of study which would undoubtedly reward further investigation in more severely affected animals.

The series of experiments attempting to explain the poor parasite establishment observed in the previous study revealed that the parasite strain in question appeared to be of normal pathogenicity, that ammonium molybdate and sodium sulphate did not reduce the activity of the parasite, and that Suffolk x Greyface sheep aged nine months could be successfully infected with H. contortus, and were unable to mount an effective response to a vaccination regime instituted at seven months of age. Thus, the explanation for the problem encountered in the previous study remains elusive.

Turning to an entirely different aspect of the trichostrongylid parasite-host relationship, the last experiment measured the pathophysiological consequences of prolonged challenge of mature,

immune ewes with O. circumcincta larvae. It was found that the changes previously reported by Yakoob et al (1983) could persist for several months under continued larval challenge.

Previously, it has been shown that challenged, immune ewes can show a reduction in wool growth (eg. Barger et al, 1973) and milk production (Leyva et al, 1981 & 1982). Additionally, it can be postulated that the detrimental effects of infection on production may well extend to reproductive parameters, as several studies have demonstrated benefits of anthelmintic treatment on lambing percentages and birth weights (see Section 1.2). These production losses in adult animals, as with those detected in growing sheep in the haemonchosis experiments of the present study, were manifested in the absence of any conventional clinical signs of parasitic disease. They undoubtedly go largely unnoticed under normal farming conditions. Although difficult to quantify, these hidden costs of subclinical parasitism must represent a considerable economic loss to agriculture, and it may well be that they exceed the more obvious financial losses due to clinical parasitic disease.

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APPENDIX 1

Appendix to Section 3.

Appendix 1 Table 1

Serum insulin concentration of sheep infected with 350 *H. contortus* larvae/kg BWt 31 days previously (ALSI) and their pair-fed (PFSC) and *ad libitum* (ALC) controls.

Sheep No.	Serum Insulin (ng/ml)							
	8 a.m.	9 a.m.	10 a.m.	Noon	2 p.m.	4 p.m.	6 p.m.	8 p.m.
ALSI-17	0.675	0.597	0.279	0.795	0.327	0.660	0.324	0.524
ALSI-31	0.417	0.704	0.491	1.339	0.367	0.508	0.611	0.460
ALSI-7	0.634	0.773	0.761	0.849	0.981	0.860	1.128	1.153
ALSI-20	0.634	1.123	0.797	1.128	0.760	0.792	0.879	0.994
ALSI-27	0.382	0.494	0.233	0.451	0.447	0.377	0.356	0.491
ALSI Mean	0.548	0.738	0.512	0.912	0.576	0.639	0.660	0.724
(± S.E.)	(0.062)	(0.107)	(0.117)	(0.152)	(0.126)	(0.089)	(0.154)	(0.145)
PFSC-25	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PFSC-18	0.326	0.135	0.766	0.746	0.303	0.247	0.226	-
PFSC-12	0.1	0.156	0.318	0.151	0.227	0.101	0.129	0.220
PFSC-16	0.252	0.196	0.556	0.632	0.505	0.195	0.206	0.242
PFSC-23	0.671	0.688	0.834	0.797	0.830	0.462	0.301	0.569
PFSC Mean	0.290	0.255	0.515	0.485	0.393	0.221	0.192	0.283
(± S.E.)	(0.105)	(0.109)	(0.137)	(0.149)	(0.127)	(0.066)	(0.036)	(0.100)
ALC-37	0.307	0.462	0.117	0.518	0.685	0.503	0.533	0.717
ALC-41	0.906	0.238	0.362	0.228	0.544	0.294	0.272	0.404
ALC-15	0.350	0.276	0.146	0.091	0.132	0.317	0.361	0.244
ALC-3	1.195	1.212	1.031	1.045	1.305	1.576	1.051	1.214
ALC-19	0.499	0.667	0.449	0.782	0.809	0.674	0.823	1.106
ALC Mean	0.641	0.571	0.421	0.533	0.695	0.673	0.608	0.737
(± S.E.)	(0.175)	(0.178)	(0.165)	(0.175)	(0.190)	(0.236)	(0.145)	(0.189)

Appendix 1 Table 2

Serum cortisol concentrations of sheep infected with 350 *H. contortus* larvae/kg BWt 31 days previously (ALSI) and their pair-fed (PFSC) and *ad libitum* (ALC) controls.

Sheep No.	Serum Cortisol (ng/ml)							
	8 a.m.	9 a.m.	10 a.m.	Noon	2 p.m.	4 p.m.	6 p.m.	8 p.m.
ALSI-17	2.3	7.8	7.7	5.5	1.2	12.0	5.1	3.8
ALSI-31	5.4	18.8	20.2	48.1	6.5	11.7	16.9	10.4
ALSI-7	3.5	6.6	5.5	13.5	3.4	7.7	3.7	14.5
ALSI-20	18.8	4.3	3.4	36.8	6.1	11.0	3.1	0.9
ALSI-27	7.9	10.0	14.9	18.3	5.6	11.1	4.8	16.7
ALSI Mean	7.58	9.50	10.34	24.44	4.56	10.70	6.72	9.26
(± S.E.)	(2.96)	(2.50)	(3.14)	(7.84)	(1.00)	(0.77)	(2.57)	(3.03)
PFSC-25	8.8	17.2	9.8	10.9	12.0	14.4	4.3	19.3
PFSC-18	6.6	3.9	7.7	11.1	4.6	5.5	8.6	0.5
PFSC-12	5.8	9.7	12.9	30.8	10.3	6.3	20.1	5.8
PFSC-16	13.5	9.5	10.2	15.2	18.8	21.8	4.1	14.4
PFSC-23	10.9	7.0	38.3	22.2	2.4	5.5	3.0	17.9
PFSC Mean	9.12	9.46	15.78	18.04	9.62	10.7	8.02	11.58
(± S.E.)	(1.41)	(2.20)	(5.69)	(3.79)	(2.90)	(3.24)	(3.17)	(3.63)
ALC-37	12.3	24.7	16.4	17.8	2.5	21.0	2.6	27.2
ALC-41	10.3	12.7	18.5	8.4	16.9	18.7	1.6	27.5
ALC-15	26.5	7.4	12.3	8.2	10.5	6.7	3.8	14.7
ALC-3	18.4	11.2	21.7	16.1	1.2	16.3	18.2	18.4
ALC-19	14.9	13.9	16.5	8.0	18.5	12.3	14.4	20.7
ALC Mean	16.48	13.98	17.08	11.7	9.92	15.00	8.12	21.7
(± S.E.)	(2.85)	(2.90)	(1.53)	(2.16)	(3.56)	(2.53)	(3.41)	(2.50)

Appendix 1 Table 3

Serum growth hormone concentrations of sheep infected with 350 *H. contortus* larvae/kg BWt 31 days previously (ALSI) and their pair-fed (PFSC) and *ad libitum* (ALC) controls.

Sheep No.	Serum Growth Hormone (ng/ml)							
	8 a.m.	9 a.m.	10 a.m.	Noon	2 p.m.	4 p.m.	6 p.m.	8 p.m.
ALSI-17	1.25	4.14	1.71	0.65	3.73	0.69	2.67	0.69
ALSI-31	2.05	1.10	1.95	0.68	2.19	0.65	1.33	1.89
ALSI-7	1.47	0.62	1.32	1.13	0.80	2.14	0.93	1.71
ALSI-20	0.73	0.59	0.67	0.54	0.18	0.21	0.90	1.36
ALSI-27	5.47	3.19	3.52	0.62	0.62	1.55	1.85	2.06
ALSI Mean	2.194	1.928	1.834	0.724	1.504	1.048	1.536	1.542
(± S.E.)	(0.846)	(0.730)	(0.474)	(0.104)	(0.650)	(0.349)	(0.332)	(0.242)
PFSC-25	3.89	0.631	0.89	0.68	0.82	1.17	2.46	0.76
PFSC-18	38.04	1.47	9.98	29.8	4.97	2.59	36.04	0.15
PFSC-12	2.12	0.49	0.20	0.79	0.73	1.64	0.72	0.65
PFSC-16	4.54	1.24	0.47	1.80	1.13	3.06	2.22	0.82
PFSC-23	2.24	0.66	0.85	0.88	1.29	0.83	18.08	1.02
PFSC Mean	10.166	0.898	2.478	6.79	1.788	1.858	11.904	0.680
(± S.E.)	(6.98)	(0.192)	(1.880)	(5.756)	(0.802)	(0.422)	(6.814)	(0.145)
ALC-37	3.24	2.17	0.70	0.63	1.97	0.37	1.69	1.14
ALC-41	1.73	2.01	0.89	1.98	2.41	0.71	3.01	3.07
ALC-15	1.22	0.66	0.56	1.19	1.18	0.51	0.88	1.58
ALC-3	0.96	1.37	2.22	3.30	3.94	0.54	20.12	2.10
ALC-19	0.52	0.65	1.08	0.83	0.49	0.44	0.73	0.85
ALC Mean	1.534	1.372	1.09	1.586	1.998	0.514	5.286	1.748
(± S.E.)	(0.469)	(0.322)	(0.296)	(0.486)	(0.587)	(0.057)	(3.730)	(0.392)

Appendix 1 Table 4

Serum prolactin concentrations of sheep infected with 350 *H. contortus* larvae/kg BWt 31 days previously (ALSI) and their pair-fed (PFSC) and *ad libitum* (ALC) controls.

Sheep No.	Serum Prolactin (ng/ml)							
	8 a.m.	9 a.m.	10 a.m.	Noon	2 p.m.	4 p.m.	6 p.m.	8 p.m.
ALSI-17	48	128	42	37	40	31	51	60
ALSI-31	165	301	148	112	115	124	174	121
ALSI-7	72	69	49	76	66	100	62	112
ALSI-20	45	63	48	124	43	155	123	80
ALSI-27	87	153	137	194	83	185	158	165
ALSI Mean	83.4	142.8	84.8	108.6	69.4	119.0	113.6	107.6
(± S.E.)	(21.8)	(43.1)	(23.6)	(26.2)	(13.8)	(26.2)	(24.8)	(18.1)
PFSC-25	134	136	70	58	42	40	92	81
PFSC-18	128	94	98	106	71	79	165	5
PFSC-12	43	55	66	61	46	93	147	61
PFSC-16	114	61	85	145	79	82	142	127
PFSC-23	64	62	66	95	34	39	92	38
PFSC Mean	96.6	81.6	77.0	93.0	54.4	66.6	127.6	62.4
(± S.E.)	(18.2)	(15.2)	(6.3)	(16.0)	(8.7)	(11.3)	(15.0)	(20.5)
ALC-37	180	118	89	46	52	31	98	48
ALC-41	49	74	42	43	47	84	75	64
ALC-15	100	88	97	62	75	78	153	191
ALC-3	72	90	84	106	137	166	175	143
ALC-19	30	69	140	180	120	145	154	122
ALC Mean	86.2	87.8	90.4	87.4	86.2	100.8	130.0	113.6
(± S.E.)	(26.2)	(8.6)	(15.6)	(25.7)	(18.1)	(24.4)	(19.4)	(26.2)

APPENDIX 2

Appendix to Section 4.

Appendix 2 Table 1

Digestibility coefficients of lambs infected with 350 *H. contortus* larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC), measured at 33 - 39 days after infection.

Group	Sheep	DM	CP	CF	EE	Ash	NFE	OM	GE
HFI	HFI-61	0.596	0.520	0.326	0.764	0.384	0.709	0.621	0.583
	HFI-44	0.571	0.463	0.295	0.720	0.373	0.693	0.595	0.551
	HFI-55	0.569	0.503	0.313	0.773	0.280	0.688	0.603	0.572
HFC	HFC-26	0.619	0.544	0.371	0.811	0.426	0.720	0.641	0.607
	HFC-67	0.627	0.562	0.360	0.791	0.409	0.738	0.652	0.628
	HFC-56	0.591	0.486	0.361	0.647	0.403	0.705	0.613	0.569
LFI	LFI-60	0.672	0.635	0.438	0.886	0.452	0.764	0.698	0.678
	LFI-39	0.661	0.633	0.420	0.874	0.481	0.746	0.682	0.655
	LFI-36	0.654	0.670	0.423	0.865	0.437	0.731	0.679	0.655
	LFI-10	0.645	0.623	0.423	0.866	0.432	0.729	0.670	0.644
LFC	LFC-31	0.629	0.570	0.387	0.865	0.396	0.731	0.657	0.629
	LFC-63	0.638	0.622	0.386	0.827	0.374	0.739	0.669	0.632
	LFC-68	0.640	0.553	0.408	0.827	0.366	0.757	0.673	0.640
	LFC-12	0.658	0.570	0.445	0.864	0.466	0.752	0.680	0.646

Appendix 2 Table 2

Nitrogen (N) intake, faecal and urinary N losses, and N retention of lambs infected with 350 *H. contortus* larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC); measured at 33 - 39 days after infection.

Group	Sheep	N intake g/day	N in faeces g/day	N in urine g/day	N retention g/day
HFI	HFI-61	32.8	15.7	7.5	9.6
	HFI-44	31.8	17.1	7.5	7.2
	HFI-55	30.1	14.9	5.4	9.8
HFC	HFC-26	32.8	14.9	2.3	15.6
	HFC-67	31.2	13.7	9.1	8.4
	HFC-56	31.5	16.2	4.6	10.7
LFI	LFI-60	21.6	7.9	5.6	8.1
	LFI-39	22.5	8.3	5.5	8.7
	LFI-36	22.5	7.4	5.1	10.0
	LFI-10	22.4	8.5	7.3	6.6
LFC	LFC-31	18.4	7.9	4.3	6.2
	LFC-63	22.5	8.5	7.8	6.2
	LFC-68	22.5	10.1	3.1	9.3
	LFC-12	22.5	9.6	4.9	8.0

Appendix 2 Table 3

Pathophysiological studies, 25 - 38 days after infection (DAI). Haematocrit values (PCV); red cell (RCV), plasma (PV) and blood (TBV) volumes; and red cell (RBC) half-lives ($T\frac{1}{2}$) of lambs infected with 350 *H. contortus* larvae/kg bodyweight and given a high (HFI) or low (LFI) plane of nutrition; and their respective pair-fed controls (HFC; LFC).

Group	Sheep	PCV 25 DAI l/l	RCV (^{51}Cr) ml/kg BWt	PV (^{125}I) ml/kg BWt	TBV ml/kg BWt	$T\frac{1}{2}$ RBC (^{51}Cr) h
HFI	HFI-37	0.220	13.1	47.6	60.7	99.9
	HFI-73	0.255	15.1	45.1	60.2	133.1
	HFI-7	0.245	18.2	56.2	74.5	140.4
HFC	HFC-15	0.34	21.6	45.8	67.4	217.7
	HFC-70	0.285	22.1	51.6	73.7	215.1
	HFC-20	0.305	22.0	56.1	78.2	249.6
LFI	LFI-16	0.225	14.7	47.3	62.0	117.6
	LFI-5	0.255	14.8	48.1	62.9	139.0
	LFI-76	0.200	10.9	44.3	55.2	84.5
LFC	LFC-42	0.355	23.8	47.8	71.6	300.8
	LFC-71	0.325	20.0	47.0	67.0	263.6
	LFC-17	0.270	18.2	50.7	68.9	286.6

Appendix 2 Table 4

Pathophysiological studies, 25 - 38 days after infection. Red cell (RBC) losses into the gastrointestinal (GI) tract per day, and per parasite present per day, and the RBC equivalent of ^{51}Cr excreted in urine, measured with ^{51}Cr-labelled RBC, of lambs infected with 350 *H. contortus* larvae/kg bodyweight and given a high (HFI) or low (LFI) plane of nutrition; and their respective pair-fed controls (HFC; LFC). Figures are daily mean (± S.E.)

Group	Sheep	RBC loss into GIT ml RBC/day	Blood loss per parasite ml/day	^{51}Cr excretion in urine ml RBC/day
HFI	HFI-37	61 (2.0)	0.054	35 (1.5)
	HFI-73	28 (1.9)	0.028	29 (1.3)
	HFI-7	31 (2.5)	0.058	32 (2.0)
HFC	HFC-15	4.0 (1.1)	-	78 (9.8)
	HFC-70	0.9 (0.1)	-	41 (3.0)
	HFC-20	0.4 (0.1)	-	36 (2.7)
LFI	LFI-16	35 (1.7)	0.052	33 (3.0)
	LFI-5	23 (0.8)	0.034	28 (2.1)
	LFI-76	39 (1.6)	0.047	23 (1.4)
LFC	LFC-42	0.2 (0.1)	-	38 (3.9)
	LFC-71	0.7 (0.1)	-	30 (3.8)
	LFC-17	0.4 (0.1)	-	29 (2.3)

Appendix 2 Table 5

Ferrokinetic studies, 25 - 38 days after infection. Serum Iron (Fe), plasma iron half-life ($T\frac{1}{2}$) and the rate of plasma iron turnover (PIT) of lambs infected with 350 H. contortus larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition; and their respective pair-fed controls (HFC; LFC).

Group	Sheep	Serum Fe µmol/l	$T\frac{1}{2}$ ^{59}Fe mins	PIT µmol/kg BWt/day
HFI	HFI-37	16	38.3	198
	HFI-73	24.5	40.9	270
	HFI-7	28	79.2	198
HFC	HFC-15	32	122.3	120
	HFC-70	37	137.4	139
	HFC-20	34	91.9	207
LFI	LFI-16	25.5	36.3	329
	LFI-5	27.5	42.4	311
	LFI-76	23	36.6	278
LFC	LFC-42	28.5	140.8	97
	LFC-71	31.5	98.9	149
	LFC-17	28	116.7	121

Appendix 2 Table 6

Ferrokinetic studies, 25 - 38 days after infection. Daily gastrointestinal (GI) loss and reabsorption of haemoglobin iron (Hb-Fe) in lambs infected with 350 *H. contortus* larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition; and their respective pair-fed controls (HFC; LFC).

Group	Sheep	^{51}Cr		^{59}Fe		Intestinal Reabsorption of Hb-Fe µmol/d
		GI blood loss ml/d	Hb-Fe loss µmol/d	GI blood loss ml/d	Hb-Fe loss µmol/d	
HFI	HFI-37	302	1192	326	1287	- 95
	HFI-73	106	609	104	597	+ 3
	HFI-7	137	647	142	671	- 24
HFC	HFC-15	12	83	33	227	- 144
	HFC-70	3	18	15	91	- 73
	HFC-20	1	6	23	147	- 141
LFI	LFI-16	167	749	185	830	- 81
	LFI-5	93	473	102	518	- 45
	LFI-76	198	853	207	891	- 38
LFC	LFC-42	1	7	40	292	- 285
	LFC-71	2	14	15	102	- 88
	LFC-17	2	11	25	144	- 133

Appendix 2 Table 7

Albumin metabolism studies, 25 - 38 days after infection. Serum albumin concentration; intravascular (CA), extravascular (EA) and total (TA) albumin pools and the EA/CA ratio; plasma albumin half-life ($T\frac{1}{2}$), and the fractional rate of plasma albumin catabolism (k) of lambs infected with 350 *H. contortus* larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC).

Group	Sheep	Serum albumin g/l	CA g/kg BWt	EA g/kg Bwt	TA g/kg Bwt	EA/CA	$T\frac{1}{2}$ h (^{125}I)	k
HFI	HFI-37	31	1.48	1.87	3.35	1.87	170	0.199
	HFI-73	31.5	1.42	1.73	3.15	1.73	221	0.127
	HFI-7	27	1.52	1.78	3.30	1.78	175	0.165
HFC	HFC-15	36	1.65	3.11	4.76	3.11	334	0.145
	HFC-70	35.5	1.83	2.81	4.64	2.81	278	0.097
	HFC-20	34.5	1.94	2.43	4.37	2.43	348	0.085
LFI	LFI-16	32	1.51	1.73	3.24	1.73	193	0.140
	LFI-5	26	1.25	1.75	3.00	1.75	282	0.120
	LFI-76	29.5	1.31	1.52	2.83	1.52	186	0.187
LFC	LFC-42	37.5	1.79	2.19	3.98	2.19	458	0.088
	LFC-71	32	1.50	2.17	3.67	2.17	382	0.094
	LFC-17	36	1.83	2.45	4.28	2.45	336	0.092

Appendix 2 Table 8

Weight (Jt Wt) and composition of the right best-end neck joint of sheep infected with 350 *H. contortus* larvae/kg BWt 42 days previously and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC).

Group	Sheep	Jt Wt g	% bone	% fat	% total lean	% eye muscle	DM coeff eye muscle
HFI	HFI-61	343	28.1	22.8	49.2	19.1	0.262
	HFI-44	432	23.3	19.9	56.8	24.8	0.252
	HFI-55	396	24.3	22.0	53.7	21.3	0.249
HFC	HFC-26	371	30.5	15.4	54.1	22.8	0.248
	HFC-67	266	16.1	28.8	55.1	26.7	0.264
	HFC-56	395	24.3	21.9	53.8	21.9	0.242
LFI	LFI-60	318	24.1	14.7	61.2	29.9	0.242
	LFI-39	328	25.7	21.6	52.7	23.3	0.252
	LFI-36	285	19.4	27.2	53.4	21.3	0.229
	LFI-10	314	32.0	18.3	49.7	19.6	0.227
LFC	LFC-31	357	29.3	20.9	49.8	19.8	0.247
	LFC-63	293	28.3	17.5	54.2	23.5	0.258
	LFC-68	324	20.5	27.7	51.8	21.9	0.250
	LFC-12	312	26.1	22.1	51.8	24.8	0.233

Appendix 2 Table 9

Body composition of lambs infected with 350 *H. contortus* larvae/kg BWt 42 days previously and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC).

Group	Sheep	Bodyweight kg	Empty Bodyweight kg	Total body fat kg	Body water (% in fat-free empty body)	Total body water kg	Body protein (% in fat-free empty body)	Total body protein kg
HFI	HFI-61	38.5	29.7	5.26	73.6	18.0	20.0	4.89
	HFI-44	41.5	31.9	5.20	75.4	20.1	18.8	5.02
	HFI-55	43.5	33.8	5.52	74.5	21.1	19.9	5.62
HFC	HFC-26	39	30.7	4.88	75.7	19.6	19.9	5.13
	HFC-67	37	27.9	4.85	74.0	17.0	20.3	4.67
	HFC-56	38	28.8	5.10	72.2	17.1	21.3	5.04
LFI	LFI-60	35	27.1	4.55	75.8	17.1	19.5	4.40
	LFI-39	36	28.0	4.78	75.8	17.6	19.6	4.55
	LFI-36	36.5	28.7	4.73	74.6	17.9	19.0	4.56
	LFI-10	39.5	29.3	4.78	76.2	18.7	19.0	4.67
LFC	LFC-31	31.5	26.0	4.94	75.7	15.9	20.3	4.27
	LFC-63	35.5	28.3	4.83	73.9	17.3	19.7	4.63
	LFC-68	37	29.5	6.06	71.5	16.8	20.1	4.70
	LFC-12	40	29.3	4.91	74.0	18.0	20.0	4.89

Appendix 2 Table 10

Gain in bodyweight (BWt), empty body, protein, fat and energy over 42 days in lambs infected with 350 *H. contortus* larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC).

Group	Sheep	Gain (kg)					Gain (MJ)
		BWt	Empty body	Protein	Fat	Water	Energy
HFI	HFI-61	7	5.1	0.84	1.70	2.21	89
	HFI-44	8.5	6.2	0.77	1.46	3.68	81
	HFI-55	8.5	6.7	1.10	1.55	3.77	81
HFC	HFC-26	9	7.2	1.28	1.49	4.41	72
	HFC-67	6	3.7	0.69	1.34	1.48	71
	HFC-56	5.5	3.5	0.86	1.42	0.88	83
LFI	LFI-60	4.5	3.2	0.49	1.10	1.73	45
	LFI-39	4.5	3.4	0.51	1.22	1.80	49
	LFI-36	3.5	3.0	0.31	0.98	1.43	61
	LFI-10	5	2.5	0.22	0.86	1.61	35
LFC	LFC-31	2	2.9	0.49	1.61	1.02	71
	LFC-63	3.5	3.4	0.52	1.21	1.34	72
	LFC-68	4.5	4.2	0.52	2.37	0.54	110
	LFC-12	5	2.2	0.37	0.93	0.74	53

Appendix 2 Table 11

Fat, protein and water content, and energy value, of 1 kg gain in empty body, and % feed conversion and apparent retention of dietary energy and crude protein over 42 days in lambs infected with 350 *H. contortus* larvae/kg BWt and given a high (HFI) or low (LFI) plane of nutrition, and their respective pair-fed controls (HFC; LFC).

Group	Sheep	Fat g/kg	Protein g/kg	Water g/kg	Energy MJ/kg	Feed Conversion %	Energy Retention %	Protein Retention %
HFI	HFI-61	332	165	433	17.5	11.6	8.7	9.9
	HFI-44	235	125	594	13.0	14.6	7.6	8.8
	HFI-55	231	165	563	12.0	13.9	7.5	12.4
HFC	HFC-26	207	178	612	10.0	14.7	7.0	15.0
	HFC-67	363	187	400	19.2	9.9	6.6	7.8
	HFC-56	405	245	251	23.7	9.4	7.7	9.6
LFI	LFI-60	345	152	541	14.0	11.1	6.3	8.2
	LFI-39	381	149	529	14.4	11.0	6.8	8.4
	LFI-36	328	103	477	20.5	8.5	8.5	5.1
	LFI-10	343	88	644	14.0	12.2	4.8	3.7
LFC	LFC-31	557	170	352	24.5	5.1	10.2	8.5
	LFC-63	356	153	394	21.3	8.5	10.0	8.6
	LFC-68	565	123	129	26.2	11.0	15.2	8.6
	LFC-12	423	169	336	24.0	12.2	7.3	6.2