STUDIES ON THE PATHOGENESIS OF OVINE FASCIOLIASIS AND SCHISTOSOMIASIS

BY

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SUMMARY

The pathogenesis of the disease caused by the liver fluke, Fasciola hepatica, and the blood fluke, Schistosoma mattheei, were studied in sheep.

The relationship between host nutrition and fascioliasis was investigated by comparing the course of the disease, firstly, in animals given the same number of F. hepatica metacercariae and fed rations containing 6% or 13% crude protein and, secondly, in chronically infected sheep transferred from high to low protein diets.

In the first experiment, it was found that sheep on the lower protein ration experienced more rapidly developing anaemia and hypoalbuminaemia, more severe losses in body weight and died earlier than their better fed counterparts. Since, the fluke burdens were comparable in both groups it was concluded that the advantages displayed by the latter reflected their greater capacity to withstand the parasites' pathogenic effects rather than a superior ability to limit infection. This was supported by the results of the second experiment demonstrating a faster development of disease in infected sheep when switched from high to low protein diets.

The development of the anaemia and hypoalbuminaemia in these animals was studied using radioisotopic techniques. An excessive expansion of plasma volume was responsible for the early reductions/

reductions in PCV's and serum albumin levels in heavy infections but the principle cause of the major reductions was the biliary haemorrhage arising from the feeding activities of the adult flukes. Although the hypervolaemia occurred at an earlier stage of the infection in protein-restricted sheep, the ultimate severity of the decline in PCV and serum albumin level depended on the ability of the host to replace the blood constituents lost into the gut, and this feature was improved by the provision of a better diet. The impairment to the erythropoietic response of the infected sheep on the poorer plane of nutrition was found to result from a deficiency of iron.

The worm-free controls used in these studies were "pair-fed" to infected sheep, and this system showed that inappetence, although not the only factor, was the major cause of the weight loss following infection. Nitrogen balance measurements revealed that a reduction in the protein content of the body made up some of the loss in liveweight resulting from factors other than inappetence. The excessive nitrogen excretion was also found to occur in the urine and not in the faeces as might have been expected from the enteric blood loss. The measurement of water balance revealed that the water intake of sheep increased following infection and there was a tendency for animals to retain water particularly during the later stages of the disease. The apparent digestibility coefficients determined for a number of dietary constituents suggested that F. hepatica has no effect on the digestive function of sheep.

Suffolk/

Suffolk x Border Leicester sheep exposed to S. mattheei cercariae were found to exhibit almost no clinical signs of disease during the 12 months following infection. This finding was in marked contrast to the acute infections reported by other workers using similar levels of exposure and was all the more remarkable in view of the large numbers of worms and eggs present in the tissues ofthese sheep at necropsy. There was, however, an absence of parasite eggs in the faeces of these sheep and a lack of intestinal bleeding; an observation which implies that the major factor in the aetiology of the severe disease in sheep is the passage of eggs through the bowel wall and its associated haemorrhage.

The parasite used in this study had originated from a strain of S. mattheei which had produced acute experimental schistosomiasis in Romney Marsh sheep some years previously. In addition to the fact that different breeds of sheep were used in the two studies the parasite had also been maintained in the laboratory during the intervening years by passage through hamsters. To determine the factor responsible for this reduced pathogenicity Romney Marsh sheep were infected with 10,000 cercariae of either the hamster-passaged parasite or a strain which had been exclusively passaged through sheep. The "sheep" strain proved lethal within 13 weeks of infection whereas the "hamster" strain produced a symptomless infection. Twice as many worms of the "sheep" strain were recovered at necropsy and these worms not only produced many more

eggs/

eggs, but larger numbers were excreted in the faeces of their hosts. Clearly, "attenuation" of the parasite had taken place as a result of hamster passage.

In a further study, it was shown that prior exposure of sheep to the relatively non-pathogenic "hamster" strain of parasite largely protected them from the manifestations of acute schistosomiasis which developed in worm-free sheep also challenged with the virulent "sheep" strain of S. mattheei. The results showed that this was partly due to a reduction in the establishment of the challenge worm population but the main factor was probably a reduction in the fecundity of these worms.

GENERAL INTRODUCTION

Internal parasitic infections of livestock represent one of the largest problems facing man in his attempt to meet the growing food requirements of the modern world. Much information is now available on many of the major parasitic diseases and outbreaks of parasitism causing widespread mortalities amongst domestic animals are generally rare, due primarily to improved diagnosis and effective methods of treatment. Nevertheless, infections with parasitic worms are common in nearly all sheep and cattle causing varying degrees of morbidity and also representing a potential reservoir for more severe consequences in the event of a breakdown in the control methods at present employed. Only with a better understanding of the parasites themselves and the pathogenic mechanisms which take place within the host can man hope to overcome parasitic infections completely or at least reduce their incidence to an acceptable level. The work described in this thesis was carried out in an attempt to explain more fully the pathophysiological changes which occur in sheep following their infection with either Fasciola hepatica or Schistosoma mattheei.

Infection of sheep and cattle with the liver fluke, F. hepatica, is widespread throughout the world and a high incidence in British livestock has been shown by a number of slaughterhouse surveys. For instance, Ross[1] found 88% of cattle in Northern Ireland suffered some degree of fascioliasis, whilst in a Glasgow slaughterhouse from 1965 to 1967, 30 - 42% of cattle livers and 12 - 14% of sheep livers were found to be affected[2]. In a more recent British survey which included abattoirs in areas where fascioliasis was not previously recognised as a problem, Froyd[3] reported that 21% of all cattle

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and 7% of all sheep livers examined were found to be affected. Workers from Shell Chemicals Ltd., estimated from data collected by the Meat and Livestock Commission in 1971 that annual liver losses due to fascioliasis in Britain were valued at £2 million for cattle and £15,000 for sheep[4].

Economic losses due to fascioliasis are not restricted to liver condemnations since in addition to mortalities and cost of treatment, F. hepatica has been reported to cause reductions in liveweight gain [5 - 8], reduced milk production in cattle [9,10] and poor wool growth in sheep [11, 12].

The fact that fascioliasis poses a serious problem to the livestock industry is clearly reflected by the large amount of research activity which has gone into the study of the disease particularly over the last 20 years. The clinical features of fascioliasis in cattle and sheep following both natural and experimental infections are now well documented. Two main types of the disease are generally recognised, the acute and chronic forms. The acute form results from a large number of immature flukes migrating through the liver and is less common than the chronic form which is associated with smaller numbers of adult parasites situated in the bile ducts. The major clinical signs are depression, inappetence, weakness and pallor of the mucous membranes, with sudden death commonly occurring in the acute form whilst the chronic form is generally typified by a wasting syndrome.

Early work on the pathogenesis of fascioliasis was largely based on changes in haematology and blood biochemistry and although anaemia and hypoproteinaemia were universally recognised as major features/

features of the chronic disease there was quite a divergence of opinion as to their aetiology. It was not until the advent of suitable isotopic techniques for labelling red cells and plasma proteins that the cause of these disturbances was verified. Infected animals were found to be in a hypercatabolic state with respect to red cells and plasma proteins due to a loss of blood into the gastrointestinal tract resulting from the feeding activities of the adult flukes in the bile ducts. The isotopic work also showed that erythropoiesis was increased by the parasitised animals in an attempt to replace the red cells lost via enteric haemorrhage and suggested a similar situation occurred with respect to plasma proteins.

The extent of the current knowledge of F. hepatica infections is comprehensively covered in the reviews of Sinclair[13], Boray[14], Reid and others[15] and Dargie[16].

It is a widely held belief that the nutrition of the host animal has a direct bearing on the severity of the disease caused by parasitic infections, with a "well-fed" animal being less severely affected than one on a poorer diet[17 - 20]. Such a situation with regard to a number of helminth parasites is generally supported by experimental evidence, although a number of workers have reported that a deficiency of a specific nutrient in a diet, for example a vitamin, mineral or amino acid may have an adverse effect upon the parasite[21 - 25]. There is very little information available on the interrelationship of fascioliasis and the dietary status of the host, and since animals which become infected with F. hepatica are likely to undergo some degree of nutritional stress either as

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a result of a reduction in appetite caused by the parasite or because the disease generally occurs during the winter months when pasture growth is sparse and of poor quality, it was considered appropriate to carry out a detailed study.

The study was conducted from two standpoints, and in both cases the nutritional status of the host animals was taken to be represented by the amount of crude protein eaten daily. Firstly the effect of similar numbers of F. hepatica metacercariae on sheep maintained on different levels of crude protein intake, and secondly the effect of a sudden lowering of crude protein intake on sheep with established adult fluke populations. The results of the study are presented in the first 4 chapters of the thesis.

In chapter 1 the general clinico-pathological findings are presented, which include the clinical and parasitological findings, and haematological, serum protein, body weight and food intake changes.

In chapter 2 the haematological findings described in the first chapter are examined in more detail along with the results of blood volume, gastrointestinal blood loss and ferrokinetic measurements carried out using ^{125}I-labelled albumin and ^{59}Fe. These later measurements provide a more dynamic picture from which a better understanding of the aetiology of the haematological changes can be established.

Similarly, the measurements of albumin pool sizes, catabolic rates of albumin, and gastrointestinal plasma protein losses using ^{125}I-labelled albumin and ^{51}Cr-labelled plasma proteins were used

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to assess the development of hypoalbuminaemia in the parasitised animals and how it was affected by the diet, (Chapter 3).

In Chapter 4 an attempt was made to examine the body weight changes which had been recorded in the study. Changes in the body content of nitrogen and water were monitored using conventional balance techniques, and the apparent digestibility coefficients of a number of dietary constituents were determined at intervals following infection.

During the course of this work an opportunity arose to carry out studies on sheep infected with S. mattheei. The adult S. mattheei worm lives in the mesenteric blood vessels of the host, and it was considered appropriate to compare the pathogenesis of the disease caused by this blood fluke with that caused by the liver fluke.

Schistosomiasis has been recognised as a disease of domestic ruminants in Africa for many years, with S. mattheei being found in the Southern and Central regions of the continent while the morphologically similar species S. bovis predominates in the Northern and Eastern areas [26]. S. mattheei was discovered by Veglia and Le Roux[27] in 1926 and has since been isolated from domestic livestock in South Africa, Bechuanaland, Rhodesia, Zambia, The Congo, Mozambique, Tanzania, Malawi and Kenya [27 - 33].

Despite the fact that S. mattheei has been known to infect sheep and cattle for some 50 years it is only relatively recently that it has become recognised as an economically important parasite [34]. The reason for this is thought to be that under the pressure of increasing agricultural production with higher stocking rates and intensive/

intensive grazing systems the levels of infection to which animals are exposed has also increased. In addition the need for better grazing has meant the more widespread use of irrigation with a consequential increase in the number of sites suitable for the intermediate snail host to breed.

An abattoir survey carried out in Rhodesia in 1960 revealed that adult schistosomes were present in 70% of the cattle examined [32], and in areas of South Africa, Pitchford [35] estimated the incidence to be in the region of 80%. In view of the apparently increasing importance of schistosomiasis as a disease of domestic livestock it is essential that a more comprehensive understanding of the host-parasite relationship is available. This is particularly relevant since the control of schistosomiasis is difficult, relying primarily on the destruction of the intermediate snail host, with no reliable and effective anthelmintic available for the treatment of infected animals [26].

The disease was first described in terms of the clinical and pathological changes observed in naturally infected animals and later in experimental infections [27, 36 - 38]. The major clinical features are emaciation, inappetence, anaemia, hypoalbuminaemia and haemorrhagic diahorrea. The main pathological lesions are found predominantly in the liver, intestines and lungs, and occur in response to the deposition of eggs within the tissues.

The importance of the passage of eggs into the lumen of the gut was clearly shown by Preston and his co-workers [38 - 43] to be a prime factor in the aetiology of many of the clinical manifestations

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of acute and sub-acute schistosomiasis. Although the investigations carried out by these workers provided invaluable information on many aspects of schistosomiasis, the acute form of the disease which they studied is not as common in the field as the chronic condition associated with prolonged infection. For this reason it was considered appropriate to study in some detail the sequential changes which occur in long term infections and the results of an investigation designed along these lines are presented in Chapter 5 of this thesis. Sheep exposed to two different levels of S. mattheei infection were studied over a period of 12 months using standard clinical and parasitological parameters along with more detailed measurements of albumin and red cell kinetics.

Unexpectedly the sheep studied in the above experiment exhibited only very minor functional disturbances despite the establishment of significant numbers of adult worms. In view of this finding a further experiment was carried out in an attempt to establish the cause of the lack of pathogenicity in the infective material. Two groups of sheep of a different breed to those used previously were exposed to the cercariae of either the non-pathogenic strain of S. mattheei or a strain of the same parasite recently imported from South Africa which was known to be pathogenic to sheep. The effects were monitored clinically, parasitologically and pathophysiologically over a period of 13 weeks and the results are presented in Chapter 6.

Since the present methods of treatment and control of schistosomiasis are unsatisfactory, much interest is being shown in the possibility of immunological control. The existence of a strain

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of S. mattheei which develops fully into sexually mature adults and yet induces apparently no pathogenic effect, has obvious potential in studies involving re-infection and immunological surveillance and was considered worthy of investigation. An experiment was therefore designed to see if prior exposure of sheep to the non-pathogenic strain would afford any protection against a challenge with a virulent strain. The results of this study are presented in Chapter 7.

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GENERAL MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Sheep of three breeds were used:

Scottish Blackface

Lambs were purchased when approximately 1 week old, housed indoors on concrete with straw bedding, and fed whole milk (½ pint/ 5 lb body weight/day). Hay and lamb weaner pellets (British Oil and Cake Mills Ltd., Renfrew, Scotland) were introduced during the third week and after weaning 7 days later, a pelleted concentrate diet (British Oil and Cake Mills Ltd., Renfrew, Scotland) was fed at the rate of ½ lb/15 lb body weight/day together with hay and water ad lib. At 8 weeks of age male lambs were castrated and all lambs docked and innoculated with a combined clostridial sheep vaccine (Covexin 8, Burroughs Wellcome and Co., Berkhamsted, England). A booster dose was given 4 weeks later.

Romney Marsh

Animals of this breed were reared parasite-free in a manner similar to that described for the Scottish Blackface sheep.

Suffolk x Border Leicester

Wether lambs of approximately 12 months of age were purchased commercially and drenched with Thiabendazole (Thibenzole, Merck, Sharp and Dohme Ltd., Hoddesdon, Hertfordshire, England) at the rate of 100 mg/kg to remove gastrointestinal worms.

MAINTENANCE OF ANIMALS DURING EXPERIMENTS

Animals were normally housed in covered pens with concrete floors and straw bedding. When complete and separate collections

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of urine and faeces were required, male animals were fitted with faecal collection bags and housed in standard metabolism cages.

In some experiments a system of paired feeding was adopted. This involved pairing an infected sheep on a body weight basis to an uninfected control animal and thereafter offering the latter the same amount of food as that eaten by its partner the previous day. In all other experiments food was offered ad lib., to both infected and control animals. Unless otherwise stated water was freely available at all times.

PARASITOLOGICAL TECHNIQUES

Infection Procedures

Metacercariae of Fasciola hepatica encysted on cellophane (supplied by Dr. J. Armour of the Wellcome Laboratories for Experimental Parasitology, University of Glasgow or Dr. C.B. Ollerenshaw of the Central Veterinary Laboratories, Weybridge) were enclosed in filter paper and administered orally. To ensure swallowing the inoculum was followed by a drench of water.

Snails, Bulinus (Physopsis sp.) infected with miracidia of Schistosoma mattheei were kindly supplied by Dr. M.J. Taylor of the London School of Hygiene and Tropical Medicine and by Dr. J. Van Wyk, Veterinary Research Institute, Onderstepoort, South Africa. To maximise cercarial shedding the snails were kept in the dark for 24 hours prior to harvesting, when they were placed in a beaker containing a small amount of distilled water and exposed to strong artificial light for 4 - 5 hours. The cercarial suspension was thoroughly mixed by gentle agitation and the cercariae present/

present in 0.5 ml aliquots counted on a squared microscope plate after staining with Lugol's iodine. Prior to infection a foreleg of each sheep was closely clipped, the hoof pared and any extraneous material removed from between the clits. The whole leg was well washed with warm water and the animal restrained in a "begging" position by a seated assistant. The leg was then immersed up to the elbow for 30 min in 300 ml cercarial suspension contained in a strong polythene bag.

Faecal Egg Counts

The basis of the method used for counting S. mattheei ova was described by Pitchford and his co-workers[1]. 10g faeces were collected and homogenised with 2 ml 5% formalinised saline. Using a strong jet of water the slurry was progressively washed through sieves of 240, 95 and 50 microns respectively. The eggs and a minimum of debris retained in the latter sieve were placed in a glass vessel and diluted to 50 ml with water. 7 ml of acid Fuchsin dye was added, the solution heated to 70 - 75^{o}C in a water bath and centrifuged at 950g for 5 min. The supernatant was discarded and the sediment washed successively with solution of NaOH (1.26 g/litre) and HCl (o.88 ml conc HCl/litre). The washed sediment was then diluted to 50 ml with water and duplicate 5 ml samples of the evenly distributed suspension placed on lined glass microscope slides. The number of red stained eggs in each aliquot represented the eggs present in 1 g of faeces.

Necropsy Procedures

Animals infected with F. hepatica were slaughtered either by captive bolt pistol after which the carcase was bled out, or

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by rapid intravenous injection of Euthatal (May and Baker Ltd., Dagenham, Essex). The abdomen was incised along the ventral mid-line and the liver along with the common bile duct and part of the duodenum removed and weighed. The gall bladder was removed, the bile poured into a glass container and any flukes present retained. The liver was sliced into strips about half an inch thick, each of which was squeezed and any flukes present removed and placed in saline. After each slice of liver had been treated in this fashion it was placed in a polythene backet containing warm saline in an attempt to release any flukes which had not been detected on the first occasion. After 1 hour the strips of liver were removed from the saline, squeezed once again and discarded. The sediment left at the bottom of the saline solution was examined for the presence of flukes. After collection the flukes were counted and measured individually.

The sheep infected with S. mattheei were given an intravenous injection of heparin (10,000 - 15,000 International Units) to minimise clotting and this was followed 5 min later by Euthatal which killed the animal and anaesthetised the worms. The skin and abdominal wall were removed from an area between the dorsal and ventral mid-lines, and also between the front and back legs on the right side. The thoracic cavity was opened by removal of the posterior half of the rib cage and the exposed organs observed and any abnormalities noted. The dorsal aorta was carefully dissected out and clamped off as far forward as possible. Through an incision made in the centre of the exposed aorta, an endotracheal tube,/

tube, attached through a peristaltic pump to a reservoir containing perfusion fluid*, was inserted towards the diaphragm and firmly secured.

The posterior vena cava was clamped just above the diaphragm, and a sawn-off Pasteur pipette connected to a suction pump via a collection vessel inserted into the portal vein close to the liver. The liver and gastrointestinal tract were massaged as the perfusing fluid was pumped into the aorta, and the perfusate containing the adult worms collected into urine glasses, a procedure which was continued until the perfusate became clear. The gut from the pyloric sphincter to the rectum was removed, separated from the mesenteries and samples of each region together with snips of liver, spleen, kidney and lung tissue taken for histological examination. Any residual worms were dissected out, the remainder of each organ weighed and finally stored at -5^{o}C for tissue egg counts. Worms recovered from each animal were bulked, washed in physiological saline, stored in a refrigerator overnight and finally fixed in 5% formalinised saline for counting.

Tissue Egg Counts

Tissue digestion and egg counting were based on the method described by Taylor and co-workers[2]. 5 g snips of tissue were digested at 37^{o}C for 16 hours in 50ml digestive fluid (1% HCl and 1% pepsin or 0.4% NaOH) and after thorough agitation, 5 x 0.1 ml samples were placed in a Sedgewick Rafter counting chamber, the eggs counted and the total egg load of each organ calculated.

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* Perfusion fluid: 150g Trisodium citrate; 86g Sodium chloride and 15 ml Euthatal diluted to 10 litres with warm tap water.

BLOOD ANALYSES

Collection and Storage of Samples

Blood samples were collected from a jugular vein into evacuated glass tubes (Vacutainer, Becton and Dickinson, New Jersey, U.S.A.). Tubes containing 100 International Units of heparin as anti-coagulant were used for all haematological examinations. For analyses involving serum, clotted blood samples were left standing overnight at room temperature in inverted tubes and the serum transferred by means of a Pasteur pipette into plastic vials which were immediately frozen and stored at -5°C.

Packed Cell Volume (PCV)

Packed cell volume percentages were determined by the micro-haematocrit method. Capillary tubes containing the blood sample were sealed at one end by heat or plasticine, centrifuged for 5 min in a microhaematocrit centrifuge (Hawksley and Sons, Ltd., London, England) and the percentage PCV determined from the scale on a Hawksley Microhaematocrit Reader.

Red Cell Count (RBC)

Total red cell counts (x $10^6/mm^3$) were determined using an electronic particle counter (Coulter Model "D", Coulter Industrial Sales Co., Elmhurst, Illinois, U.S.A.).

Haemoglobin Concentration (Hb)

Blood haemoglobin was estimated by the cyanmethaemoglobin method[3]. 0.02ml of well-mixed blood were added to 5 ml dilute potassium ferricyanide solution. Haemoglobin thus oxidised to haemiglobin was converted by potassium cyanide to the stable cyanmethaemoglobin. This compound was measured colorimetrically at/

at 542mμ on an SP600 spectrophotometer (Pye Unicam, Cambridge, England) and the haemoglobin concentration (g/100 ml) determined with the aid of standard cyanmethaemoglobin solution (Roche Diagnostics, Roche Products Ltd., London, England).

Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC)

These indices were calculated as follows:

$$\text{MCV } (\mu^3) = \frac{\text{PCV x 10}}{\text{RBC}}$$

$$\text{MCHC } (\%) = \frac{\text{Hb x 100}}{\text{PCV}}$$

Serum Iron

Blood samples collected in iron-free tubes were allowed to clot and the serum obtained treated first with an anionic detergent (Teepol, in acetate buffer, pH 5.8) to split the Fe^{3+} transferrin complex and subsequently with sodium dithionite to reduce free Fe^{3+} to Fe^{2+}. Addition to bathophenanthroline disulphate (7.5 mmol/litre) produced a reddish-pink complex, the colour intensity of which was read on a spectrophotometer at 546 mμ. Serum iron concentration was calculated by reference to the colour intensity of a standard solution treated as above.

Serum Proteins

Total serum proteins were estimated by a biuret colorimetric technique[4], and albumin by the bromocresol green method described by Rodkey[5]. Serum globulins were calculated as the difference between total protein and albumin concentrations.

COLLECTION OF URINE AND FAECES AND SAMPLING OF FEEDSTUFFS FOR CHEMICAL ANALYSES

Individual faecal and urinary outputs and feed intakes were recorded daily throughout balance experiments. Ten per cent samples of the daily faecal outputs were retained (stored at $4^{o}C$) and combined, and at the termination of each period faecal slurries were prepared by homogenising 100 g aliquots of well-mixed bulked samples with water and toluene (5 ml). The remainder of the sample was analysed for moisture content and hammer-milled for further analysis.

Urine was collected in plastic buckets containing 100 ml M-H_2SO_4 and 10% of each daily output was retained and combined over the balance period. This bulked sample was then filtered (Whatman No. 1 filter paper, W. and R. Balston Ltd., England) and 100 ml of the filtrate retained for analysis.

Random samples (totalling about 500 g) of each consignment of compound feedstuff were collected and hammermilled prior to analysis. When chopped hay was fed, random handfuls of each batch were similarly treated. Since some animals tended to be very selective when offered hay it was often necessary to collect the material left at the end of a balance period and treat it as described for a hay sample in order to obtain a more accurate measure of intake.

CHEMICAL ANALYSES

Dry Matter

Samples of known weight were dried in a forced-draught oven at $100^{o}C$ to constant weight.

Organic Matter and Ash

Samples of dried material were weighed into a crucible, charred using a bunsen burner and placed in a muffle furnace at 550 - 600°C for 5 hours. After cooling in an evacuated desiccator and re-weighing organic matter and ash contents were calculated as the loss of weight and weight of residue respectively.

Ether Extract (Fat)

Estimation of ether soluble material was performed by extraction of feed and faecal samples for 4 hours in a Soxhlet apparatus using petroleum ether (B.P. 40 - 60°C).

Nitrogen (Crude Protein)

Total nitrogen analyses were carried out by a macro-Kjeldahl technique[6].

Faeces and Feed

Approximately 7 g faecal slurry (or 1 - 2 g hammermilled feed) were weighed accurately into a Pyrex glass thimble which was then dropped into a 500 ml long-necked Kjeldahl flask. 25 ml sulphuric acid, together with 2 copper Kjeldahl catalyst tablets (BDH Chemicals, Poole, England) and a few boiling chips were added and the mixture boiled until the solution cleared. Gentle boiling was continued for a further 30 min after which the contents of the flask were made alkaline by addition of 40% sodium hydroxide solution. The ammonia was then distilled into 40 ml 2% boric acid solution containing 4 drops methyl red/methylene blue indicator, and the green boric acid/ammonia solution titrated with hydrochloric acid (HCl) of known molarity until returning

to/

to the red colour present prior to distillation. Given that 14g nitrogen is equivalent to 1 litre M-HCl and that 1 g nitrogen corresponds to 6.25 g crude protein, the nitrogen and crude protein contents /_ g slurry and hence / · g faecal dry matter can be calculated.

Urine

These were determined in a manner similar to that described for faeces except that 10 ml samples were digested with 10 ml sulphuric acid.

Iron Content of Feed

5 ml conc sulphuric acid (analar) and 4 ml conc nitric acid (analar) were added to an accurately weighed sample of feed (about 100 mg), heated at 200°C for 2½ hours in a sealed teflon bomb, and after cooling 5 ml hydrofluoric acid (analar) were added and the mixture reheated for a further hour. The resultant solution was then transferred when cold to a 100 ml volumetric flask containing 3 g boric acid and diluted with distilled water. The iron concentration of this solution was then determined by flame atomic absorption spectrometry (Model 306, Perkin-Elmer Ltd., Beaconsfield, England).

DETERMINATION OF APPARENT DIGESTIBILITY COEFFICIENTS (ADC %)

These were calculated for dry matter, organic matter, ash, ether extractable material and crude protein by substituting the relevant data in the following equation:

$$\text{ADC \%} = \frac{(\text{Amount in feed} - \text{Amount in faeces}) \times 100}{\text{Amount in feed}}$$

RADIOISOTOPIC TECHNIQUES

Labelling of Red Cells with ^{51}Cr

The successful labelling of red cells with ^{51}Cr depends upon the fact that anionic hexavalent chromate penetrates the cells, is reduced to cationic trivalent chromium (Cr^{3+}), and becomes bound to the globin moiety of haemoglobin [7, 8]. Hence the procedure in general consists of incubating a volume of the animal's blood or red cells *in vitro* with a suitable amount of radio-chromium as hexavalent chromate (e.g. Na_2CrO_4). Since excessive amounts of chromium can damage the cells it is recommended that the specific activity of the ^{51}Cr should be less than 2 µg chromium/ml of packed red cells, equivalent to approximately 0.7 µg/ml whole blood[9].

The procedure adopted in the present studies involved collecting blood (equivalent to about 5 ml of packed red cells) by jugular puncture into universal bottles containing heparin as anticoagulant. After centrifugation for 10 min at 550 g and removal of plasma, the cells were suspended in isotonic saline and gently mixed. A measured volume of isotonic saline containing 1 mCi ^{51}Cr as sodium chromate (specific activity 250 µCi/µg Cr, Radiochemical Centre, Amersham, England) was added with gentle mixing. The labelled cell suspension was incubated at 37°C for 30 min with frequent gentle mixing and then centrifuged at 550 g for 10 min. The supernatant was removed and discarded, the cells washed in isotonic saline until free of unbound ^{51}Cr, and finally reconstituted with the retained plasma for injection. In all cases, each sheep received its own erythrocytes and plasma.

Labelling of Plasma Proteins with ^{51}Cr

Plasma proteins were labelled in vivo by intravenous injection of 1 mCi ^{51}Cr as chromic chloride (specific activity 150 μCi/μg Cr, Radiochemical Centre).

Labelling of Plasma and Red Cells with ^{59}Fe

These were labelled in vivo by intravenous injection of ^{59}ferric citrate (specific activity 15 μCi/μg, Radiochemical Centre) corresponding to a radioactivity of approximately 50 μCi.

Labelling of Albumin with ^{125}I

Trace-labelling of proteins with radioiodine was carried out by the iodine monochloride method of McFarlane[10]. This method depends upon treating the protein in slightly alkaline solution with iodine monochloride to which has been added the radioactive iodine as carrier-free iodide (obtained from the Radiochemical Centre as thiosulphate-free $Na^{125}I$) and results in substitution of ^{125}I in the tyrosine residues to give mono and di-iodo tyrosine groupings. The introduction of less than 1 atom of iodine/molecule of albumin ensures that no marked change occurs in its physiochemical and immunological properties[11].

Materials

(i) Albumin

Commercial sheep albumin (Cohn Fraction V, Pentex, Incorp., Kankakee, Illinois, U.S.A.) was used in all experiments.

(ii) Iodine Monochloride

A solution containing 0.42 mg I/ml as iodine monochloride (ICl) in M-NaCl and approximately 0.01 N with respect to HCl was used./

used. This was prepared by dissolving 5.00 g potassium iodide (KI) and 3.22 g potassium iodate (KIO_3) in 37.5 ml distilled water. To this was added 37.5 ml concentrated HCl and 5 ml carbon tetrachloride (CCl_4) and the mixture shaken vigorously; 0.1 M-KI was then added dropwise until a faint pink colour appeared in the CCl_4. This stock solution, which contains approximately 147 mg I/ml as ICl was diluted 1 in 350 with isotonic saline to provide a solution containing 0.42 mg I/ml.

(iii) Glycine buffers

Two glycine buffers were used for the labelling process. Buffer A (pH 8.5) was used for conversion of ICl to hypoiodite (IOH), while buffer B (pH 9.0) was employed for solution of the protein at an alkaline pH.

Buffer A: 45 ml M-glycine in 0.25 M-NaCl + M-NaOH to pH 8.50.

Buffer B: 40 ml M-glycine in 0.25 M-NaCl + M-NaOH to pH 9.00.

Procedure

A 2% solution of sheep albumin was prepared by dissolving 600 mg freeze-dried protein in 30 ml isotonic saline and buffered by addition of 15 ml glycine buffer B. 10 mCi of carrier-free radioiodine was added to 2.5 ml of a freshly prepared solution of ICl containing 0.42 mg I/ml; the iodine monochloride solution was then converted to hypoiodite by addition of 10 ml glycine buffer A and immediately mixed with the protein solution. The labelled preparation was transferred to a dialysis sac containing 2 g "carrier" protein (bovine serum albumin). Carrier protein

was/

was added to reduce the specific activity of the labelled albumin to less than 5 μCi mg, thereby reducing the possibility of radiation decomposition[11, 12]. The labelled protein was dialysed for 48 hours at 5°C against two 20 litre changes of isotonic saline to remove unbound iodide and finally centrifuged for 30 min at 550 g prior to injection.

Labelled albumin prepared in this way contains 0.9 atoms/ molecule assuming 100% incorporation.

Injection of Radioisotopes

All radioactive materials were injected into a jugular vein through a jugular catheter (Portex Plastics Ltd., Hythe, England), and the catheter flushed out with isotonic saline before being withdrawn.

Radioactivity Measurements

1 ml samples of blood and plasma were pipetted into counting bottles and made up to 15 ml with 0.02 M-NaOH. The volume of each daily urine collection was measured and a 15 ml aliquot taken for radioassay. Each 24-hour faecal collection was weighed and mixed thoroughly and random 15 g samples packed in counting tubes. Radioactivity measurements were carried out using an automatic well-type gamma scintillation spectrometer (Nuclear Chicago, High Wycombe, England). Standard solutions of all labelled preparations were assayed at regular intervals and corrections for radioactive decay based on the activities of these solutions. In a number of experiments reported in this thesis radioactivity due to three isotopes ^{59}Fe, ^{51}Cr and ^{125}I was measured simultaneously. Separation of isotopic mixtures was achieved by conventional γ ray spectrometry and where necessary/

necessary the application of "overlap" factors calculated from the relative count rates of standard solutions of these isotopes at each photopeak.

STATISTICAL METHODS

Statistical methods employed were those described by Bishop[13]. Half-life values quoted in the test were calculated by regression analysis and unless otherwise stated, correlation coefficients of radioactivity against time were very highly significant (r -0.95). Standard errors (SE) and p values (student's t-test) are quoted. A p value equal to or less than 0.05 is regarded as being statistically significant.

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CHAPTER 1

The Relationship of Host Nutrition and Fascioliasis: The clinico-pathological changes in sheep following infection with F. hepatica

INTRODUCTION

Two forms of ovine fascioliasis are described in most parasitological textbooks[1,2]. The most spectacular of these is acute fascioliasis, brought about by the ingestion of large numbers of metacercariae over a short period and characterised by sudden death due to the severe abdominal haemorrhage and liver damage caused by immature flukes migrating through the hepatic parenchyma. The second and more common form of disease, chronic fascioliasis, is associated with the presence of adult flukes in the bile ducts causing a progressive wasting condition which frequently culminates in death. While division of the disease into these types is useful if only for its simplicity, rarely can infections be described in such clear-cut terms; for this reason two further classifications, "sub-acute" and "subclinical" have been proposed. The former, which is intermediate between the acute and chronic forms arises from the intake of large numbers of metacercariae over a prolonged period and is characterised by the presence of both adult and immature parasites in animals which develop severe clinical signs during the 2 - 3 weeks preceding death[3,4]. Subclinical fascioliasis, which occurs in association with an adult fluke burden insufficient to cause overt disease is claimed to impair productivity, but the importance of this form is stressed more by drug firms selling anthelmintics than by parasitologists.

The/

The clinical effects of acute fascioliasis have been described by a number of authors [3 - 7], the main featuresbeing inappetence; death, usually occurring within 8 - 9 weeks of infection; anaemia, which is generally normocytic and normochromic; hypergamma-globulinaemia; severe liver damage as measured by elevated serum concentrations of liver enzymes and by reduced bromsulphthalein clearance; and accummulation of ascitic fluid. Chronic fascioliasis has been described as a condition causing inappetence, weight loss or poor weight gain, reduced wool growth, pallor of the visible mucous membranes, submandibular oedema and ascites, with death often occurring 15 - 40 weeks after infection [3, 4, 8, 9.]. In addition to these changes an initial hyperproteinaemia due to increased levels of gammaglobulin is followed by progressive hypoproteinaemia in association with reduced levels of both albumin and globulin. The anaemia which develops concurrently is initially normocytic and normochromic but progresses to one characterised by macrocytosis, and in more severe and long-standing cases, hypochromia.

Discrepancies in the onset and severity of the above disturbances are with some justification usually attributed to differences in fluke burden since throughout the spectrum of disease it is the number of parasites present which is the most striking feature. It is however widely believed that in addition to the parasite burden, the severity and ultimate outcome of many infections are determined by the quality and in particular the protein content of the diet available to the host, "well-fed" animals apparently being less affected clinically than their "poorer-fed" counter-parts.

While/

While this seems a reasonable supposition, and indeed has been clearly verified in a number of diseases caused by gastrointestinal parasites [10, 11], the part played by host nutrition in the development of ovine fascioliasis has never been examined in sufficient depth to allow any firm conclusions to be drawn. In fact there are only two reports in the literature on this aspect - one of which appears in Boray's review of experimental fascioliasis in Australia[12], while the other deals with the topic from the standpoint of wool growth [13]. Boray described an experiment where young sheep infected with 3,000 F. hepatica metacercariae and fed a low protein diet consisting largely of wheaten chaff died from acute fascioliasis after 6 - 7 weeks while animals similarly infected but fed a high protein ration containing crushed wheat, lucerne and wheaten chaff survived for 13 weeks. Unfortunately no further details were given although the author did present some preliminary data from a subsequent study indicating that poorly fed animals died earlier, became more anaemic and harboured larger flukes producing more eggs than similarly infected but better fed sheep. Roseby [13] on the other hand demonstrated that wool growth was depressed by about 30% in animals infected with about 190 adult flukes and perhaps surprisingly that this was unrelated to dietary quality.

From the foregoing account it is apparent that the information currently available regarding the role of host nutrition in the pathogenesis of ovine fascioliasis is at best scanty and from several standpoints non-existent. In view of the likelihood that affected animals suffer some degree of dietary deprivation during the course of/

of a typical fluke season - either as a result of the inappetence reputedly caused by the parasite and/or its winter prevalence, and the importance of an adequate protein intake in promoting growth and maintaining essential body functions, e.g. immunological surveillance[14], red cell [15], and protein synthesis [16], it seemed appropriate to carry out a detailed study of the influence of dietary protein on the development of this disease.

In the present investigations the effect of diet on the host-parasite relationship was examined from two standpoints. Firstly the extent to which protein intake influences the establishment and/or the pathogenic effects of the parasite. This was studied by monitoring the clinical changes arising in sheep exposed to similar levels of infection but maintained on diets providing different crude protein intakes. Secondly the consequences of a sudden lowering of nutrition in chronically infected sheep; this aspect was examined by following the development of disease in animals maintained on a high protein ration for 16 weeks after infection and subsequently fed a diet of low protein content. In both experiments the food intake of worm-free control animals was regulated to that of the parasitised sheep by a system of "paired-feeding" thereby enabling effects other than those caused by inappetence to be observed. This chapter describes the general clinico-pathological changes recorded in the course of these experiments, while the under-lying pathophysiological mechanisms are reported in subsequent chapters.

MATERIALS AND METHODS

Experimental Animals and Design

Two experiments were carried out using 6 - 9 month old Scottish Blackface wethers reared and maintained under worm-free conditions. In the first, 16 sheep were divided into two equal groups; all animals were fed chopped hay *ad. lib.*, but each member of one group was offered in addition 500g daily of a high protein compound diet (Diet 1). Six weeks later, 5 animals in each group were individually infected with 1,000 *F. hepatica* metacercariae. The remaining worm-free controls were allotted on a body weight basis to 3 infected sheep in each group and paired-feeding commenced and maintained for 20 weeks or until the parasitised animals were believed to be *in extremis*.

The second experiment involved 12 sheep, half of which were each infected with 600 *F. hepatica* metacercariae and paired by body weight with a worm-free partner. Paired feeding, which commenced 3 weeks before infection was continued until the 7th week after infection on a high protein ration (Diet 2) but due to limited supplies this was replaced by a similar diet (Diet 1) which was fed until the 16th week; thereafter the animals were offered a low protein ration (Diet 3) and this was continued until necropsy in the 20th week.

The sheep were confined in standard metabolism cages throughout and allowed free access to water. Food consumption was recorded daily, and/

and all sheep weighed and blood and serum samples for haematological and biochemical analyses collected twice weekly just prior to the morning feed.

Diets

The pelleted high protein compound Diets 1 and 2 had a basic composition of 50% chopped barley straw, 23.3% wheat feed, 7.0% ground nut meal, 2.5% barley, 10.0% molasses, 7.2% mineral and vitamin additives. Diet 1 comprised 95.0% dry matter, 15.0% crude protein, 9.5% ash and 2.2% ether extractable material, while diet 2 consisted of 89.1% dry matter, 13.6% crude protein, 10.4% ash and 2.0% ether extractable material.

The low protein diet (Diet 3) was also in a pelleted form and consisted of 80% chopped barley straw, 15% molasses and 5% mineral and vitamin additives giving on analysis 90% dry matter and 7.8% crude protein.

Chopped hay was generally of poor quality and variable composition ranging between 89.8% - 94.6% dry matter, 4.1 - 9.3% crude protein, 5.0 - 9.8% ash and 0.9 - 1.5% ether extractable material.

Blood Analyses

Packed cell volumes (PCV), total serum protein, serum albumin, globulin and albumin:globulin ratios were measured in both experiments. Determinations of red cell counts (RBC) and haemoglobin concentrations (Hb) and hence mean corpuscular volume (MCV) and haemoglobin concentrations/

concentrations (MCHC) were restricted to the first experiment as were estimations of serum glutamic oxaloacetic transaminase (SGOT) levels).

Necropsy Procedure

At death or slaughter of an infected sheep in Experiment 1, its respective feed control was necropsied so that direct comparisons could be made. The abdomen and thorax were opened, the liver removed and weighed, and the total fluke burden and individual worm lengths determined. Liver weights and fluke lengths were not recorded in the second experiment.

RESULTS

EXPERIMENT 1

Two groups of Scottish Blackface wethers were maintained on diets consisting of chopped hay only or chopped hay and a high protein compound diet. Six weeks later, 5 animals in each group were individually infected with 1,000 F. hepatica metacercariae and together with 3 worm-free "pair-fed" controls measurements of various haematological and biochemical parameters and of body weight and food intake made during the following 15 - 20 weeks when the animals were necropsied and their fluke burdens and individual fluke lengths determined. To facilitate the presentation of results only the mean values of the pair-fed sheep are depicted in the figures but some individual data are included to illustrate features of particular interest; the remainder are given in Appendix 1.

Clinical and Necropsy Findings

All infected animals developed most of the recognised symptoms of fascioliasis but in general sheep restricted to hay exhibited clinical signs and either died or were killed in extremis 3 - 5 weeks earlier than those receiving additional concentrates. Of the animals maintained on hay, four were killed in extremis around the 15th week after infection and only one survived to week 20, whereas with the exception of one animal which was necropsied during week 17, sheep fed hay plus concentrates survived until the 20th/

Table 1

Post-mortem Findings on Sheep in Experiment 1

Diet	Sheep No.	Length of Infection (days)	No. of Flukes Recovered	Fluke Length(mm) Mean	Fluke Length(mm) Range	Fresh Liver Wt. gms	Fresh Liver Wt. % Bwt
Hay.	Infected						
	989	102	576	16.14	8-25	782	2.97
	981	106	520	13.34	7-20	690	2.79
	961	106	502	16.39	7-25	720	2.76
	985	101	543	11.56	4-23	582	2.29
	992	139	354	15.90	7-27	714	2.41
	Mean	111	499	14.67		698	2.65
	S.E.	± 7	± 38	± 0.95		± 33	± 0.13
	Control						
	3	-	-	-	-	460	1.70
	6		-	-	-	540	2.10
	962		-	-	-	562	1.93
	Mean					521	1.91
	S.E.					± 31	± 0.12
	p					<0.02	<0.01
Hay + Compound Diet	Infected						
	5	139	453	17.88	7-27	920	2.96
	994	122	396	16.76	5-27	1030	2.84
	965	139	666	13.66	5-23	842	2.82
	954	139	620	15.82	4-28	1014	3.44
	4	139	515	19.72	10-30	943	2.98
	Mean	136	530	16.76		950	3.01
	S.E.	± 3	± 50	± 1.01		± 34	± 0.11
	Control						
	7	-	-	-	-	481	1.42
	952		-	-	-	508	1.63
	964		-	-	-	576	1.82
	Mean					522	1.62
	S.E.					± 28	± 0.12
	p					<0.001	<0.001

20th week when they too were killed *in extremis* (Table 1).

The main clinical signs recorded in both groups were inappetence, weight loss, weakness, pallor of the visible mucous membranes and in most cases, submandibular and facial oedema. At *post-mortem*, ascites and hydrothorax of varying degrees were constant features as was hepatomegaly which appeared to be most marked in those receiving concentrates (Table 1).

The percentage fluke recovery (Table 1) was high in both groups, ranging between 35% and 58% in the sheep restricted to hay and between 40% and 66% in those given additional concentrates, but there was no significant difference between the groups either in the total number or length of the individual flukes which became established; the small fluke burden accorded to No. 994, which of the sheep given concentrates was the most severely affected, is probably an underestimate since this animal died 3 - 4 hours before fluke recovery.

Haematological Changes

The mean haematological values recorded for the sheep on each diet are illustrated in Fig. 1. Marked anaemia first became evident in the poorer fed sheep about 4 weeks after infection when the mean PCV dropped below 25% (range 20 - 25%) and became significantly lower than the corresponding control value ($p < 0.02$). This downward trend continued unabated during the remainder of the experiment, but was most marked between the 12th and 15th weeks when average values fell from about 15% to 8% and four of the animals had to be necropsied; the/

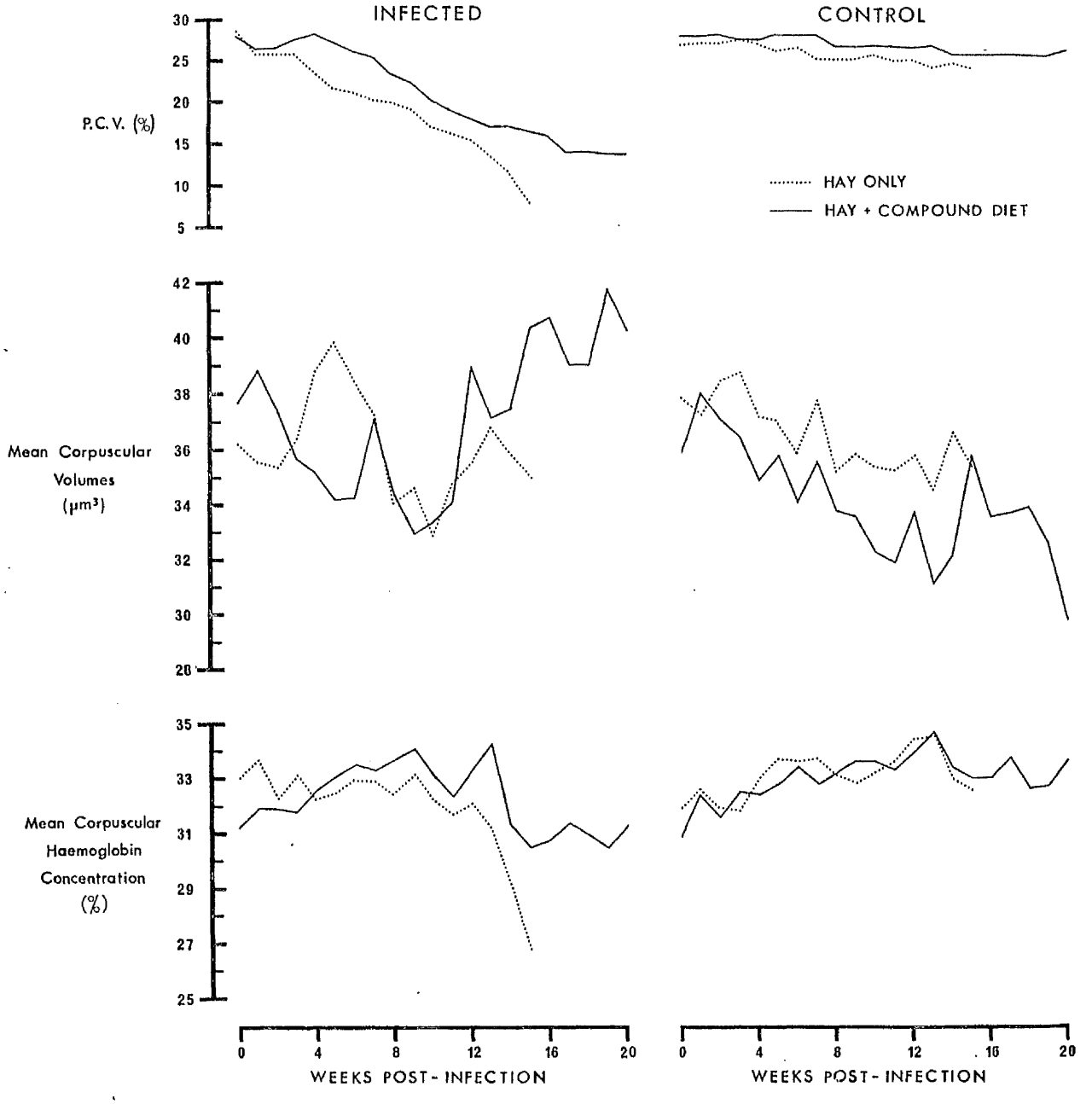

Fig. 1 : Effect of Diet on the Haematological Indices of Fluke-Infected and Control Sheep.

the one animal which survived until the 20th week had a terminal PCV of 9%.

Infected sheep fed the additional compound diet also developed severe anaemia but this was delayed in onset until about the 7th or 8th week and only became significantly lower than the control value after 10 weeks ($p < 0.02$). Between the 8th and 12th weeks the average PCV of this group deteriorated at a rate similar to that recorded for the animals restricted to hay but thereafter (with one exception which was necropsied on week 17 with a PCV of 9%) fell less rapidly; at the termination of the experiment on week 20 the mean haematocrit of the survivors was 14%.

The trends observed with regard to total red cell counts and haemoglobin concentrations were generally similar to those described for PCV but a number of differences emerged which were reflected in alterations in MCV and MCHC. The anaemia which developed in the animals receiving the additional compound diet was initially normocytic and normochromic but developed into one characterised by macrocytosis, with MCV values being consistently elevated between the 14th and 20th weeks. The poorly fed sheep in contrast not only exhibited smaller increases in MCV which were restricted to the period 4 to 8 weeks post-infection but also developed marked hypochromia terminally. Mean values for the two groups of control sheep were similar throughout the study and while MCV's generally declined, the MCHC's remained relatively constant.

Biochemical Data

The mean results of the serum protein estimations are illustrated in Fig. 2. Both groups of infected animals exhibited similar responses although a number of differences in timing and magnitude were observed. At infection the average total protein level of the sheep fed hay only, was 5.5g%, but this increased progressively from about the 2nd week, reaching 7.7g% by the 9th week; differences between infected and control values were significant throughout this period ($p < 0.05 - 0.01$). This upward trend was abruptly reversed thereafter and by the 15th week the average concentration had fallen to 4.6g%. Sheep given concentrates also developed progressive hyperproteinaemia during the early stages of infection but this was more gradual and less dramatic with total protein values rising from 6.1 to 7.2g% by the 10th week, being significantly higher than control values between the 7th and 10th weeks ($p < 0.05 - 0.01$). In common with their poorer fed counterparts this group suffered a drop in total protein level after about 11 weeks which although less rapid nevertheless resulted in terminal concentrations which were significantly lower than those recorded for the controls, i.e. 3.8 and 5.7 g% respectively ($p < 0.05$).

Serum albumin concentrations followed a downward trend in all sheep as a result of infection but like the PCV changes described earlier this was more pronounced in the poorer fed animals. Average figures for this group, which even before infection were somewhat lower/

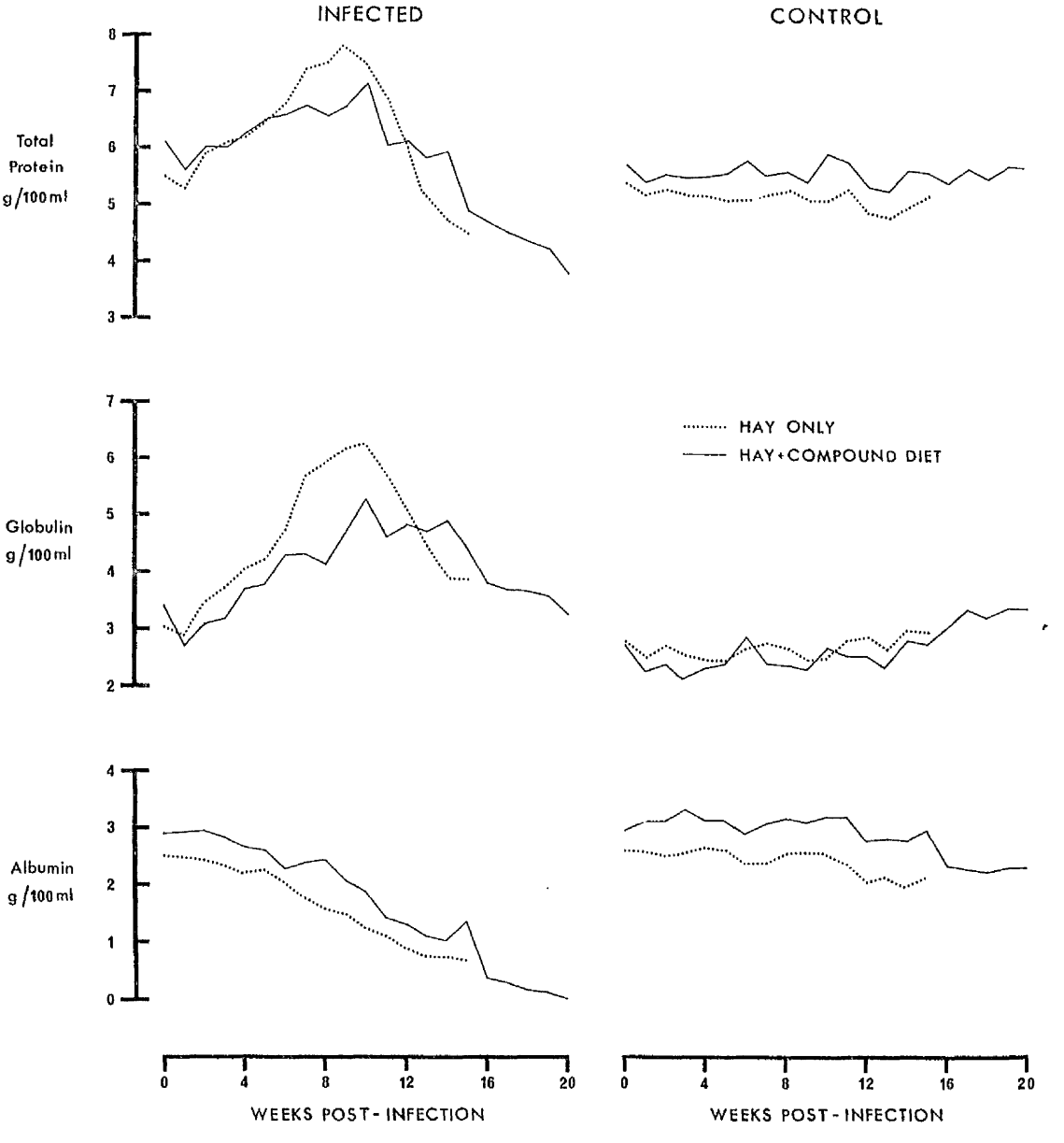

Fig. 2 : Effect of Diet on the Serum Protein Levels of Fluke-Infected and Control Sheep.

lower than those of the sheep given concentrates, fell slowly during the early stages of the disease to a level which by the 6th week constituted significant hypoalbuminaemia (i.e. 2.0g% compared to 2.5g% in the paired controls). However the deterioration in albumin status recorded subsequently was much more dramatic and by the 15th week the average concentration was 0.7g%. Control values also fell during this latter period and at necropsy ranged from 1.9 - 2.5g%. Infected sheep fed hay and compound diet were not significantly hypoalbuminaemic until the 7th week when average values had fallen to 2.4g%; however like the group restricted to hay these animals subsequently suffered a sharp drop in albumin levels which by the 20th week had fallen to 0.5g%. Control sheep, which up until the 15th week had maintained their albumin concentrations between 2.8 - 3.2g%, developed mild hypoalbuminaemia over the latter stages of the investigation.

Serum globulin changes closely paralleled those described for total protein, all infected animals showing an initial hyperglobulinaemia followed by a progressive decline to values similar to those recorded prior to infection. Differences between groups of infected sheep were not significant at any stage but in general hyperglobulinaemia was more pronounced in the sheep restricted to hay with maximum concentrations reaching 6.3g% on the 10th week as compared with 5.3g% in the group given the additional compound diet; control values were similar in all animals but appeared to increase terminally in association with the feed restriction imposed by the inappetence of their respective infected partners.

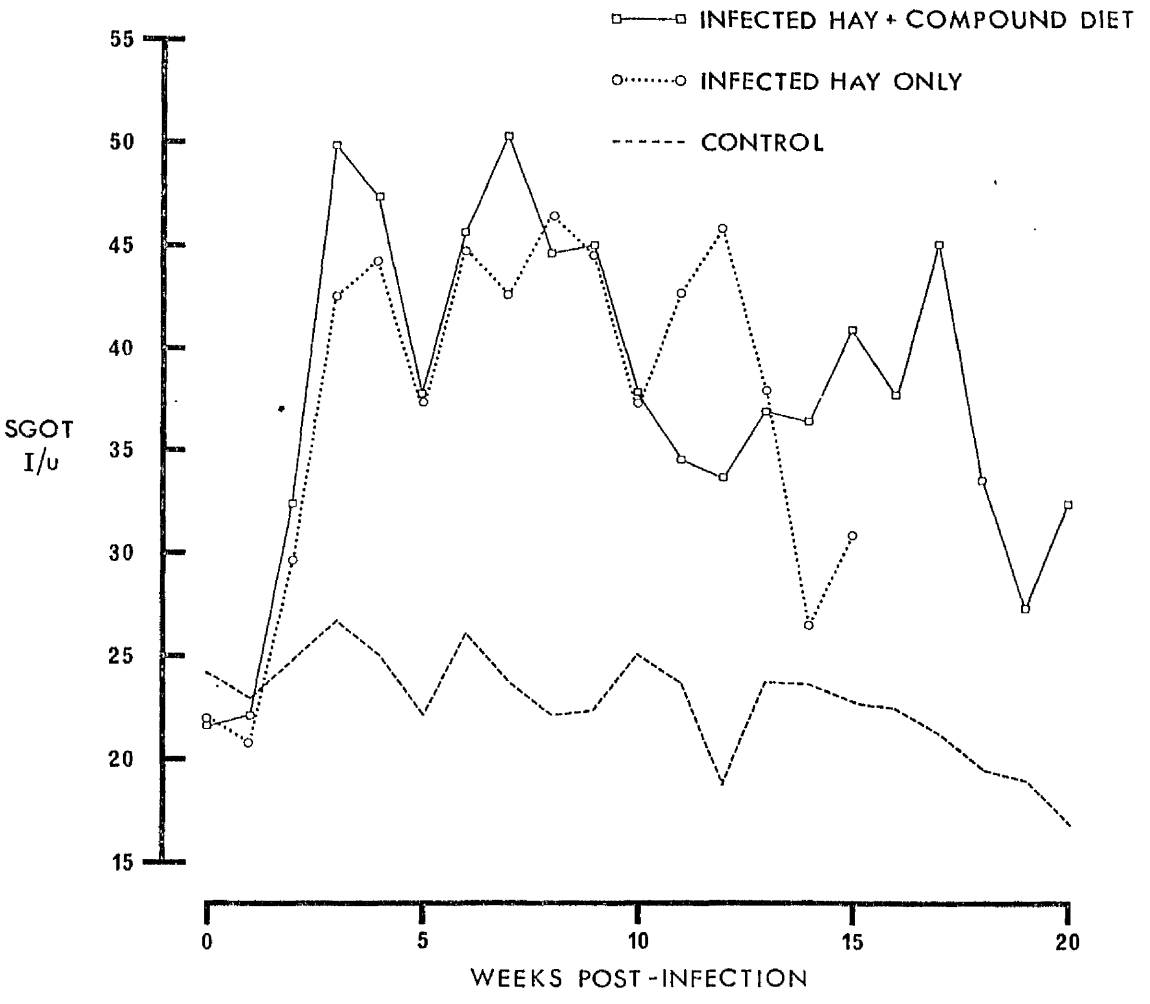

Fig. 3 : Effect of Diet on the SGOT activities of Fluke-Infected and Control Sheep.

As a result of these changes albumin : globulin ratios decreased steadily in both groups of infected sheep but again this feature was more pronounced in those restricted to hay. Ratios for the infected animals on this diet fell from 0.85 to 0.18 between the 3rd and 15th weeks whereas control values, which were well maintained at around 0.95 until the 12th week, declined thereafter and by week 15 averaged 0.70. Reduced ratios were also recorded for sheep on the higher protein ration but only at necropsy in the 20th week were these comparable with the terminal values recorded 5 weeks earlier in their poorer-fed counterparts.

The mean serum glutamic oxaloacetic transaminase (SGOT) activities of the pair-fed sheep are depicted in Fig. 3; since both groups of worm-free sheep had comparable values throughout the study the activities illustrated for the controls represent the average value of all animals. Infected sheep on each plane of nutrition exhibited comparable and highly significant elevations in SGOT between the 2nd and 12th weeks after infection ($p < 0.05$ - 0.001) and although the values in the majority declined to control levels thereafter, transient increases in a few individuals resulted in the average value remaining high.

Body Weights, Dry Matter and Crude Protein Intakes

The average daily intakesof dry matter and crude protein together with the weekly body weights are illustrated in Fig. 4.

Despite/

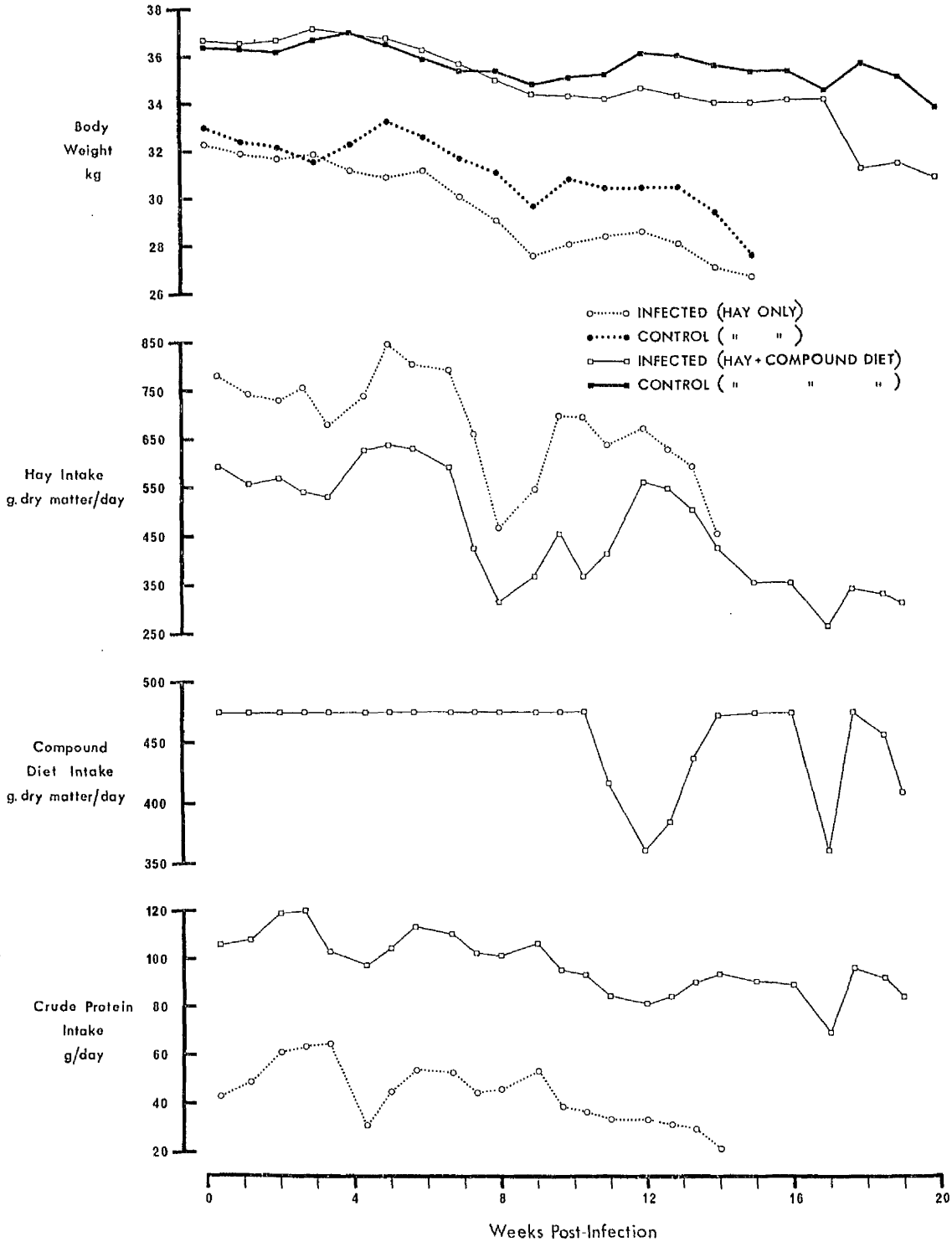

Fig. 4 : Body Weights and Feed Intakes of Fluke-Infected and Control Sheep.

Despite considerable day-to-day variations in appetite, and even more pronounced differences in the crude protein content of individual batches of hay which gave rise to divergent patterns of dry matter and crude protein intakes, a number of inter-relationships emerged between the various parameters measured.

Sheep restricted to hay maintained fairly steady body weights (31 - 32kg) and showed no loss of appetite during the first 6 weeks of infection; over this period the average daily dry matter intake fluctuated between 700 - 800g and provided about 50g crude protein (range 30 - 60g). Depressed feed intake was first detected around the 7th week and became more pronounced over the following 2 - 3 weeks when dry matter consumption fell to about 500g/day and liveweight to 27.5kg. Following a transient improvement between the 9th and 12th weeks, appetite again deteriorated and in some cases approached almost complete anorexia prior to death or necropsy in the 15th week; by this stage dry matter and crude protein intakes had dropped to 450g/day and 23g/day respectively, but body weights were relatively well maintained at about 27.0kg.

Although the controls showed similar weight changes some divergence was noted from the 4th week and by week 9 infected sheep were significantly lighter than controls ($p < 0.02$). This difference was either maintained or became more pronounced as the infection progressed but it was noteworthy that during the two weeks preceding necropsy, and in association with the marked feed restriction enforced by the inappetence of their infected counterparts, the weights of worm-free/

worm-free sheep fell more dramatically with the result that by the termination of the experiment the total weight loss suffered by the control sheep (5.4 kg) was almost identical to that of their parasitised partners (5.6 kg).

Animals receiving hay supplemented with the compound diet showed only marginal weight gains over the first 6 weeks of the infection despite consuming more feed (approximately 1150g dry matter/day providing about 110g crude protein/day). However when their appetite for hay, like that of their poorer-fed counterparts became depressed between the 7th and 9th weeks, and dry matter intake fell to 850g/day. weight loss was minimal (1.3 kg). These animals also showed a renewed interest for hay between weeks 9 and 12, but since this was accompanied by a reduced demand for the compound diet, crude protein intake continued to decline and by the 12th week was only 80g/day With the subsequent and more or less progressive reduction in hay consumption renewed interest was shown for the concentrate ration and the average crude protein intake although falling sharply between the 16th and 17th weeks because of the almost complete inappetence of one animal, remained fairly steady at around 90g/day.

Body weights of infected and control sheep were almost identical until the 9th week. but subsequently there was a tendency for the former either to gain less or lose more weight than the latter · a feature particularly obscured in the figure by a weight increase/

increase of 0.8 kg between the 14th and 17th weeks in the animal which died prior to the termination of the experiment and by the fact that its pair-fed control lost 3.7 kg over the same period. Weight losses suffered by the other animals on this diet during the infection were 6.1 and 5.5 kg for the paired infected sheep, 2.9 and 3.9 kg for their respective controls and 4.1 and 4.7 kg for the remaining infected sheep.

When this study was initiated the average body weight of each nutritional group was about 33.6 kg; at the end of the 6 week pre-infection period, the sheep restricted to hay weighed 33.0 kg whereas those given the additional concentrates weighed 36.2 kg ($p < 0.05$). As the infection progressed, differences between groups of both infected and control animals on each diet became progressively more significant ($p < 0.02$-0.001); and by the 15th week when the sheep fed hay were necropsied a differential of about 7.5 kg was recorded.

EXPERIMENT 2

Six Scottish Blackface wethers were each given 600 *F. hepatica* metacercariae and paired to a worm-free control animal, whose feed intake was restricted to the same level as its infected partner. All sheep were maintained on a high protein ration (13-15% crude protein) during the first 16 weeks of infection and on a low protein diet (8% crude protein) for a period of 4 weeks thereafter. Haematological and biochemical determinations together with measurements of/

of body weight and feed intake were made at frequent intervals throughout and fluke burdens determined atnecropsy. In the interests of clarity only the mean values of the infected and control groups are depicted in the figures but individual data are presented in Appendix 1.

Fluke Recovery

The average percentage fluke recovery in this experiment was 33% (range 20 - 70%), representing a mean recovery of 199 ± 47 flukes. It must be stressed that since different batches of metacercariae were used in the two experiments, no significance can be attached to these differences in fluke burdens.

Haematological Changes

The average PCV values recorded during the experiment are illustrated in Fig. 5. All infected animals became anaemic from about the 6th week but this was neither marked nor significant until the 9th week when the mean PCV had fallen to 24%. This degree of anaemia persisted until the 16th week but with the change to low protein feeding became much more pronounced, PCV's falling to 17% by week 20. Control values generally ranged between 30 and 34% and were unaffected by the change of diet.

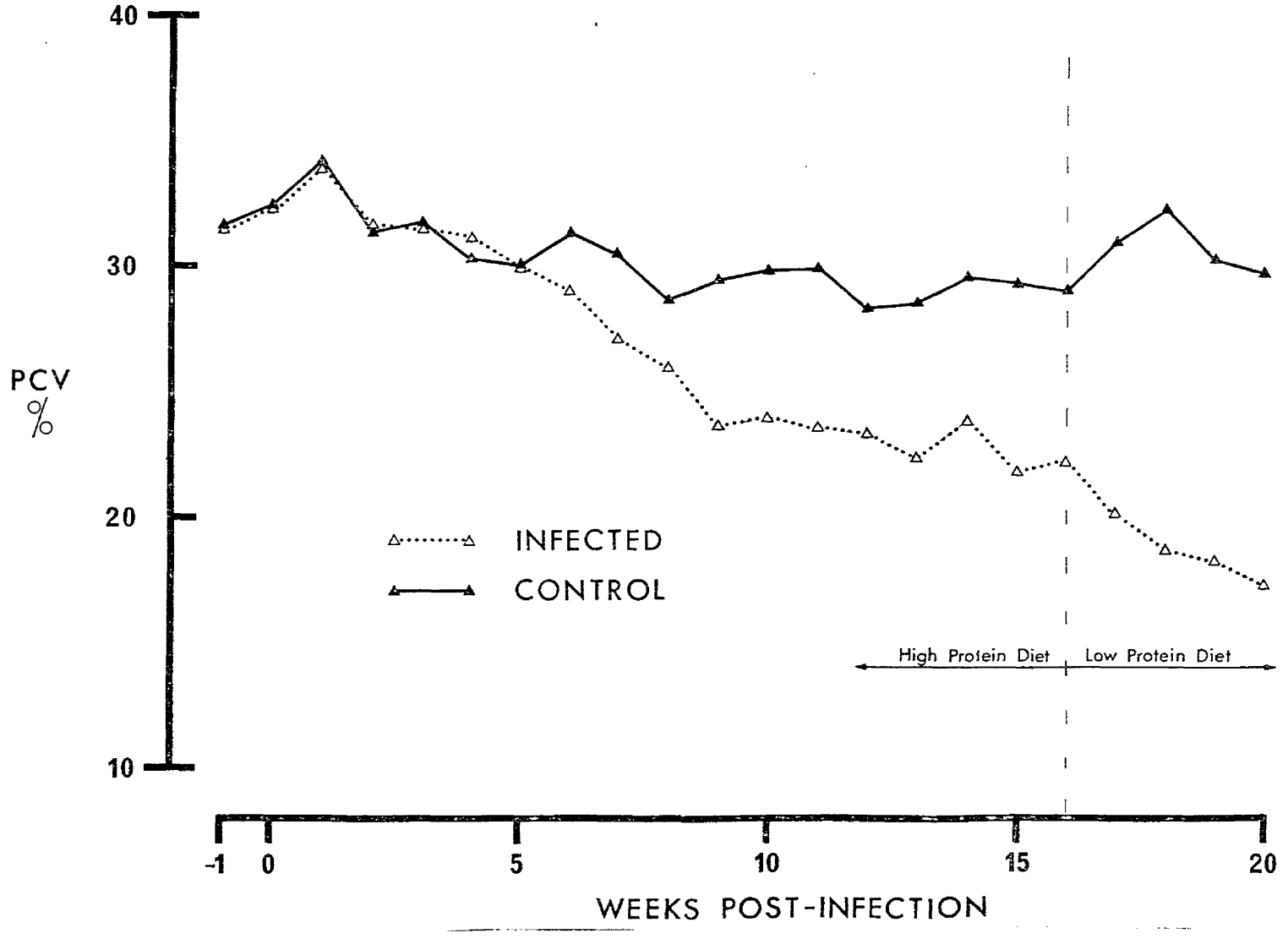

Fig. 5 : Effect of Diet on the PCV Levels of Fluke-Infected and Control Sheep.

Biochemical Data

The mean serum protein changes are illustrated in Fig. 6. Total protein concentrations increased progressively between the 3rd and 10th weeks, and following a period of relatively slow decline over the next 6 weeks deteriorated more rapidly during the subsequent 4 weeks of low protein feeding; by week 20, average concentrations which between the 5th and 11th weeks were significantly higher than normal ($p < 0.05 - 0.001$) approached levels which almost constituted significant hypo-proteinaemia (i.e. 4.9g% compared with 5.8% in the controls).

Serum albumin levels began to fall from about the 3rd week and by 8 weeks were significantly ($p < 0.05$) lower than control figures (3.0g% and 3.4g% respectively); although further reductions were apparent thereafter the rate of deterioration was markedly accelerated following the switch to low protein feeding and at necropsy average concentrations had fallen to 1.7g%; control values which until the 16th week had remained steady at about 3.5g% fell slightly thereafter and at necropsy averaged 3.2g%.

Globulin levels followed a pattern similar to that described for total protein except that control figures increased slightly following the change to the low protein diet.

As a result of their superior albumin status albumin: globulin ratios were considerably higher in these sheep than

in/

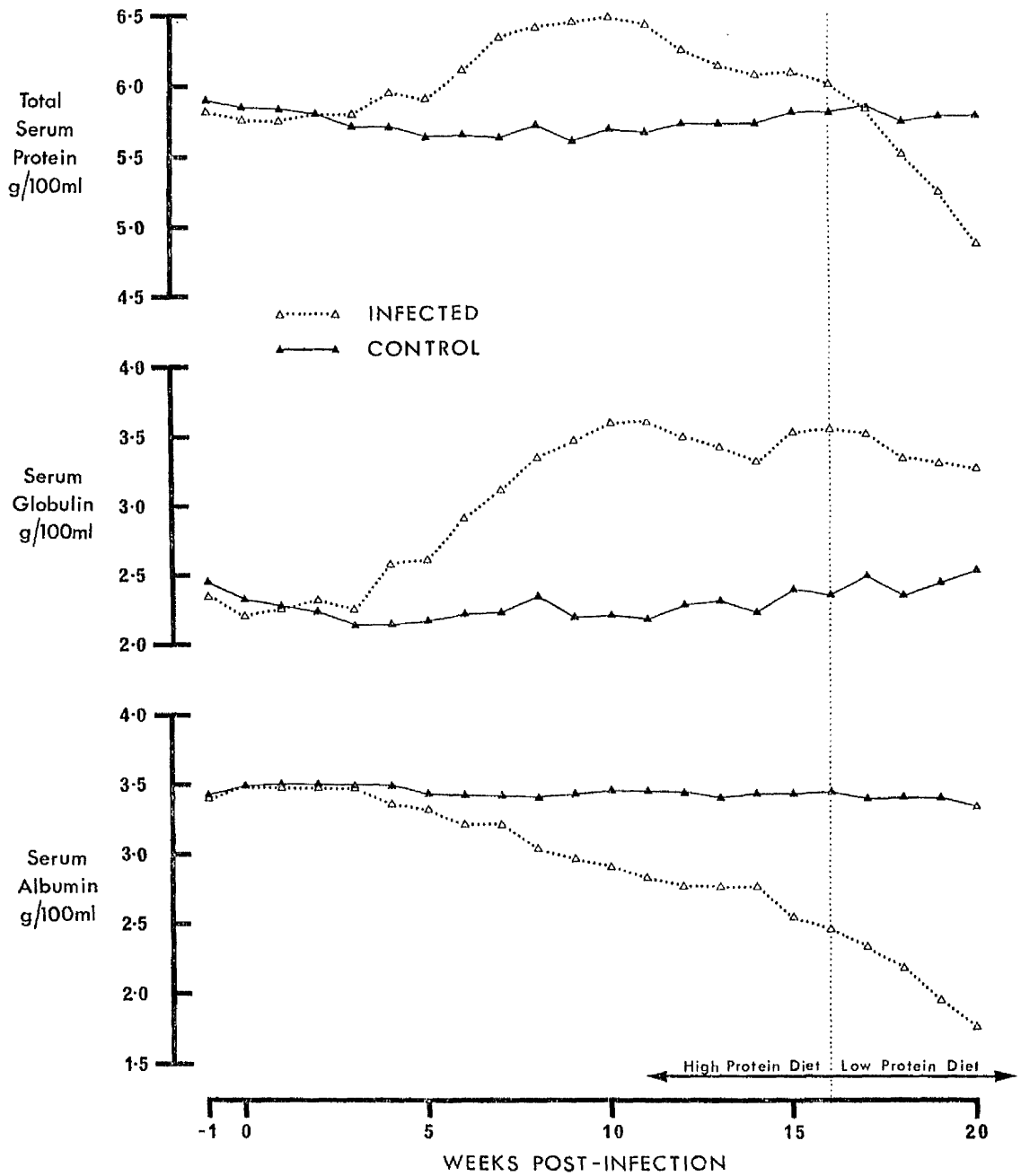

Fig. 6 : Effect of Diet on the Serum Protein Levels of Fluke-Infected and Control Sheep.

in the animals used in Experiment 1. Nevertheless, values for infected sheep fell progressively between the 3rd and 20th weeks (from 1.6 to 0.6) and control figures after fluctuating around 1.6 until the 16th week subsequently fell to 1.3.

Body Weights, Dry Matter and Crude Protein Intakes

Throughout the period of high protein feeding, body weights of both infected and control sheep increased progressively and by the 16th week of infection average weight gains of 9.5 kg and 9.3 kg respectively were recorded (Fig. 7). Following the introduction of the poorer quality diet, all animals continued to gain weight for about 1 week but thereafter suffered weight loss which was somewhat more rapid in infected than control sheep (i.e. 2 kg and 1.2 kg respectively).

The uniform composition of the compound diets used helped to reduce fluctuations in feed and hence dry matter and crude protein intakes. Only 3 of the infected sheep suffered any deterioration in appetite but this was slight (about 15% around the 8th week of infection). Throughout the period of high protein feeding, the average daily intake of crude protein never fell below 130g, and due to the introduction of another diet on the 7th week which had a slightly higher protein content, increased progressively to around 170g between the 7th and 16th weeks. With the introduction of the low-protein diet on the 16th/

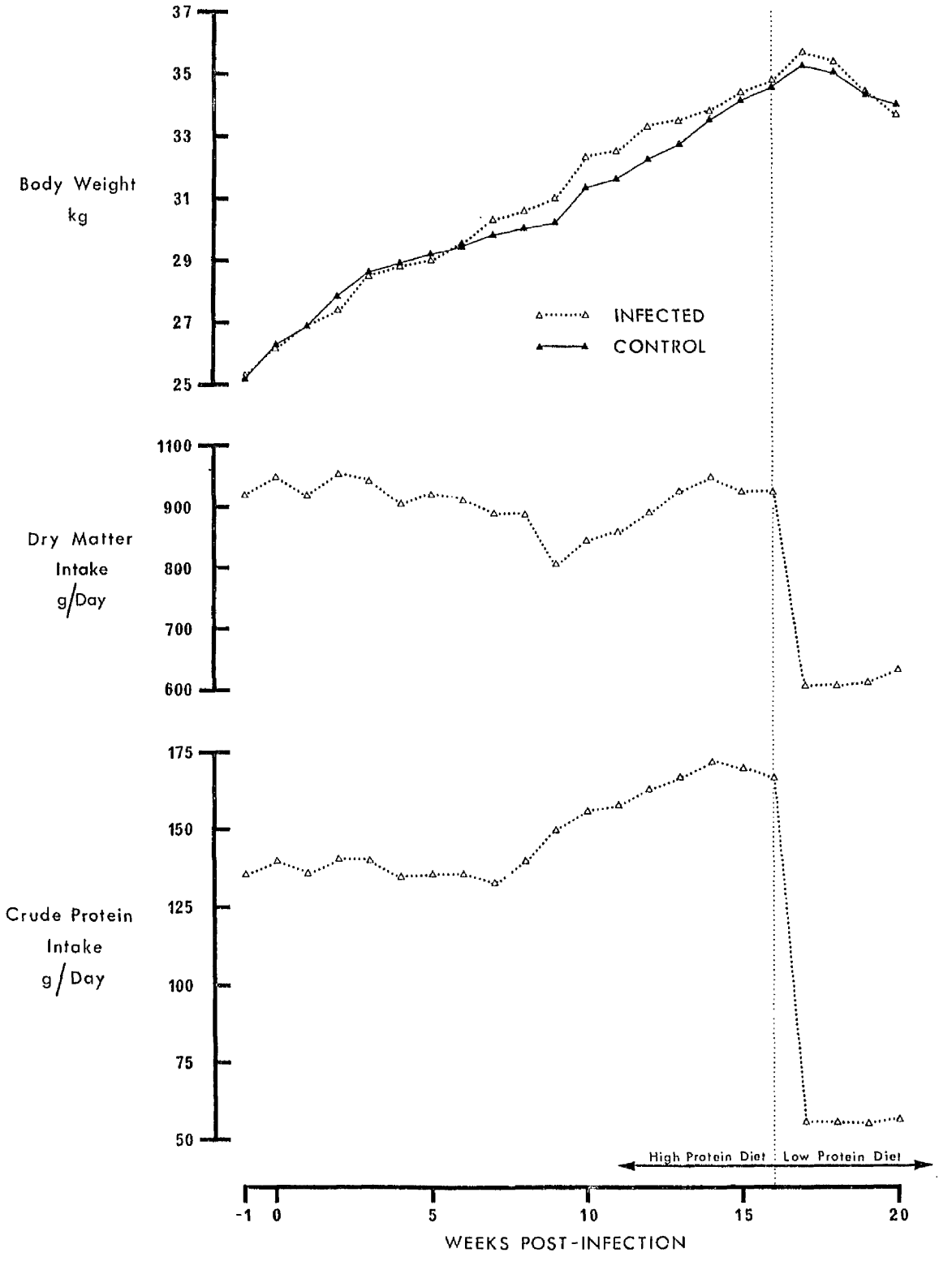

Fig. 7 : Body Weights and Feed Intakes of Fluke-Infected and Control Sheep.

16th week, appetite was markedly depressed as a result of unpalatibility and this, together with the lower quality of the diet, dramatically reduced crude protein intake to about 60g; this level was maintained over the remaining 4 weeks of the study.

DISCUSSION

Fascioliasis is best known for its adverse effect upon growth and for the fact that it causes anaemia, hypoalbuminaemia and elevated serum transaminase levels; these events, in many instances, culminate in death. The results reported in this chapter clearly demonstrate that the severity of this disease as assessed by the majority of these indices is profoundly influenced by the quality of the diet available to the host, "well-fed" animals being less affected than those on "poor" feed. This is illustrated in the first experiment by the more rapid development of clinical symptoms and earlier mortalities among infected sheep restricted to a maintenance ration (containing about 6% crude protein) as compared with animals, similarly infected, but receiving a diet commensurate with growth (13% crude protein) and in the second by the more rapid deterioration in the clinical condition of infected sheep following their transfer from high to low protein feeding.

In theory there are two possible explanations for the accelerated development of the disease in sheep fed low protein rations. The simplest is that because poor nutrition adversely affects a number of host defence mechanisms[14, 17] such animals were immunologically less competent than their better-fed counterparts and hence suffered the more severe liver damage and haemorrhage associated/

associated with the establishment of larger fluke burdens. Alternatively by virtue of their smaller size and lower metabolic reserves the poorer fed sheep were physiologically less able to counteract the pathogenic effects of their parasite populations.

If fluke burden is used as the criterion of immunological competence then it is clear from Table 1 that this function was not impaired by low protein feeding. This finding, which confirms Roseby's [13] original observation that fluke establishment is unrelated to the host's nutritional status may appear to contradict the generally stated view that poor nutrition enhances susceptibility to helminthic infections [10, 18]. While it is likely that sheep are indeed more "susceptible to infection" when maintained on protein-depleted diets, only rarely is it stated whether this reflects greater "susceptibility to establishment" or greater "susceptibility to the effects of establishment". Examination of the more recent literature reveals only two instances in which low protein feeding predisposes animals to heavier infections, i.e. the studies of Bawden [19], and Dobson and Bawden [17], demonstrating larger populations of adult Oesophagostomum columbianum in sheep fed a 7% crude protein diet than in similarly infected animals fed a ration containing 19% crude protein, and the studies of Downey and colleagues [20] illustrating a comparable, but less consistent effect with Ostertagia circumcincta. On the other hand Gordon [21] observed little difference in the establishment of Trichostrongylus colubriformis/

colubriformis between sheep fed high and low protein rations and results from earlier work [22 - 24], although often cited as favouring greater worm loads in poorly fed sheep do not withstand critical analysis since the experiments were conducted either on naturally infected sheep where intake of feed and larvae were unknown or on very small numbers of experimentally infected animals.

On the other hand if immunological competence is assessed in terms of fluke size, then the fact that the parasites recovered from the low protein group 14 -15 weeks after infection had already attained a size comparable to that found in the high protein group 5 - 6 weeks later suggests that poor nutrition, although not affecting ultimate establishment, does nevertheless predispose the host to a faster rate of fluke migration and development. Since most of the clinical effects of fascioliasis arise primarily from liver damage and biliary haemorrhage [25] the earlier and more severe disturbances experienced by the poorer fed sheep might be explained on this basis. There are, however, a number of points which do not substantiate this argument. Firstly, fluke size is very variable and only broadly related to age of infection [5, 26 - 29], and hence cannot be considered a reliable basis either for comparing fluke development or assessing their pathogenic effects. Secondly, if indeed fluke migration was accelerated by low protein feeding this was not reflected by the SGOT levels which were elevated to a similar extent and duration/

duration in both nutritional groups. Finally, differences in parasite migration or development, even if operative in the first experiment could not account for the more rapid deterioration in the clinical condition of the infected sheep used in the second, when transferred from high to low protein feeding.

It would therefore appear that parasite development was not significantly affected by the host's plane of nutrition and that the profound clinical differences observed were primarily a reflection of the physiological rather than the immunological status of the animals concerned, i.e. their ability to repair the damage caused by the haematophagic and tissue-feeding activities of the parasite. This will obviously depend to a large extent on the availability of essential metabolites to those organs involved, e.g. in haemopoiesis and regeneration of plasma and cellular proteins, which in turn will be related to the amount and the quality of diet ingested. The purpose of this section is to highlight the clinical effects observed in these studies and their relationship to fluke development and host nutrition; detailed accounts of the mechanisms responsible for these changes are given in subsequent chapters.

From the haematological data it is apparent that diet has a major influence both on the development of the anaemic process and on its morphological characteristics. Sheep receiving hay and concentrates developed significant anaemia only/

only after about the 8th week of infection; this condition was therefore associated primarily with the adult fluke population. By contrast those receiving only hay and which harboured similar fluke burdens experienced marked reductions in PCV during the migratory stages of the infection. Moreover whereas the former developed a very pronounced macrocytosis when PCV's fell below about 20% and only terminally showed signs of impending haemoglobin deficiency, (this is illustrated by the maintenance of reasonably normal MCHC values during the initial 16 - 17 weeks of infection), red cells of the latter group, with the exception of a short period between the 4th and 7th weeks of infection, remained virtually normal in size throughout and by the 12th week became grossly depleted of haemoglobin. Since macrocytosis is indicative of the presence of reticulocytes, which in turn enter the circulation in large numbers only when erythrocyte production is greatly accelerated [30], these findings are compatible with a higher rate of red cell synthesis in the better fed sheep. The reason for this difference has yet to be determined, and while it is known that protein deficiency, per se does not impair erythropoiesis directly [30], other studies have demonstrated that haemopoiesis, in rats deprived of protein was limited by a lack of the stimulating agent, erythropoietin [15, 31]. While such a mechanism could account for the apparently poorer response of the sheep fed low protein diets, in view of the attendant hypochromia, iron deficiency also remains a strong possibility.

The/

The serum protein changes recorded in these experiments were substantially the same as those described by others [4, 8, 32] and need not be described in detail. Nevertheless it is worth emphasising that each of the major changes observed, i.e. hyperglobulinaemia and hypoalbuminaemia were most pronounced in those animals with the highest fluke burdens, and within groups of similarly infected sheep, in those maintained on the poorest plane of nutrition. Since hyperglobulinaemia is generally considered to reflect the host's immune response to parasitic and/or auto antigens released during the course of fluke migration, and hypoalbuminaemia the result of excessive protein loss via the bile and possibly impaired synthesis by the liver [25], there is nothing surprising about most of these relationships. Moreover the one finding for which there is no immediate explanation is the exceptionally high globulin levels found following infection of those fed the low protein ration. This suggests that in these animals antigens were either made available in greater amounts or were more efficiently processed. While the former seems unlikely since fluke burdens were similar in both groups, it is possible that even in the absence of significantly higher SGOT levels, the livers of the protein deprived animals were more extensively damaged and hence produced larger amounts of auto antigens, or were less efficient in trapping antigen with resultant diversion to antibody-producing sites, e.g. the spleen. As yet there is little evidence for the involvement of either process in fascioliasis. Complement-fixing anti-tissue antibodies have been/

been demonstrated in infected sheep [33], but it remains to be determined whether these are actually hepatatoxic. Likewise, although it is known that hepatic fibrosis causes marked alterations in the intrahepatic vasculature of diseased animals with resultant development of portal hypertension [33], it has yet to be established whether this impairs the filtering capacity of the liver.

For sometime it has been recognised that the liver fluke frequently causes inappetence [6, 8, 12, 27, 28, 34], but the significance of this feature, which will obviously have an important bearing on the host's nutritional status, has never been examined critically. This was facilitated in the present experiments by the use of pair-fed controls in which the effects of dietary restriction per se could be observed and distinguished from other possible aetiological factors. The first point which deserves comment with regard to inappetence is that its occurrence was restricted to animals with relatively high fluke burdens (i.e. greater than about 200 flukes), and although to some extent selective, was most pronounced around the 7th to 10th weeks of infection and again during the few weeks preceding death. These findings, which are in broad agreement with those reported by Boray [12], provide no direct insight into the cause of inappetence but indirectly implicate the damage caused around the time of fluke migration into the biliary system, and latterly the presence of severe anaemia and hypoalbuminaemia. Secondly, judging by control values, inappetence was the major cause of weight loss but contributed/

contributed little to the haematological and serum protein changes which accompanied the disease; the small reductions in PCV and serum albumin experienced by the controls, especially those fed low protein diets, were largely unrelated to periods of feed restriction and probably resulted from blood sampling. Finally, since, infected sheep were more severely affected than their pair-fed counterparts, suggests that this disease developed from factors additional to decreased feed intake, but this does not exclude the possibility that inappetence potentiates the effects of such factors in parasitised sheep. This finding is by no means unique to fascioliasis since sheep infected with Trichostrongylus axei [35], Trichostrongylus colubriformis [36 - 38], Oesophagostumum columbianum [19, 39], Haemonchus contortus [40, 41], and Schistosoma mattheei [42], all develop more severe symptons of disease than worm-free animals restricted to the same level of feed intake.

With regard to the body weight changes recorded in the course of these experiments two further points emerged. Firstly, the presence of 500 flukes was sufficient to cause significant weight loss irrespective of dietary quality, but sheep fed low protein diets experienced earlier and more severe emaciation than their better fed counterparts. Secondly, under conditions of high protein feeding, neither body weight itself nor the capacity to gain weight was adversely affected by 200 adult flukes. This somewhat surprising finding is probably explained largely by the complete/

complete lack of inappetence in such animals and throws doubt on the economic significance of subclinical fascioliasis. However it must be stressed that live weight, although easily measured, is not the only index of productivity, and carcass quality, for example, which depends upon the relative proportions of body solids and water may change independently of weight. The possibility of such an occurrence, due to excessive water retention in infected animals, is suggested from the first experiment by the appearance of oedema and by the finding that despite almost complete inappetence infected sheep generally maintained their body weights.

In conclusion, these studies clearly demonstrate that dietary quality has a considerable bearing on the pathogenesis of a single experimental infection of F. hepatica, and while the available evidence strongly suggests that the clinical advantages displayed by animals on a high plane of nutrition over their poorer fed counterparts arose principally from the better capacity of the former to withstand the parasite's pathogenic effects, the possible involvement of variations in fluke migration and development cannot be discounted entirely. To clarify the relative importance of these factors requires detailed comparative information on the development of the parasite and disease under the nutritional conditions adopted, which cannot be obtained solely on the basis of clinical values since by their very nature these are merely indirect indicators of worm activity and disease severity./

severity. For a proper appreciation of how diet affects this parasite and the accompanying disease it is therefore necessary to describe the development of each in terms of the direct functional disturbances taking place within the host's tissues. This was attempted in the same animals by reference to the anaemia and the serum protein and body weight changes, and the results are presented in the following three chapters.

SUMMARY

The relationship between host nutrition and development of clinical fascioliasis in sheep was investigated by comparing the course of the disease, firstly in animals given the same number of F. hepatica metacercariae and fed rations containing 6% or 13% crude protein, and secondly in chronically infected sheep transferred from high to low protein diets.

In the first experiment, it was found that sheep on the lower protein ration experienced more rapid deterioration in their clinical indices and died earlier than their better fed counterparts. Since the fluke burdens were comparable in both groups it was concluded that the advantages displayed by the latter reflected their greater capacity to withstand the parasites' pathogenic effects rather than a superior ability to limit infection. This was supported by the results of the second experiment demonstrating a faster development of disease in infected sheep when switched from high to low planes of nutrition.

The importance of reduced appetite was also studied by comparing the clinical indices of infected sheep with those of worm-free animals maintained on the same level of feed intake. It was found that loss of appetite contributed substantially, although not entirely to weight loss, but had little effect on the attendant anaemia and serum protein changes.

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CHAPTER 2

The Relationship of Host Nutrition and Fascioliasis: The Aetiology of the anaemia which develops in sheep following infection with F. hepatica

INTRODUCTION

Many theories have been advanced to explain why sheep infected with F. hepatica develop anaemia, and most of the useful information available on this topic has been reviewed by Sinclair[1], and Dargie[2]. The main point which emerges from these reviews is the stark contrast in the conclusions reached by their respective authors regarding the basic cause of this anaemia, the former attributing it to a disorder of the reticuloendothelial system, the latter to an increased rate of red cell destruction or loss brought about by the blood sucking activities of adult parasites. In view of this discrepancy it is relevant to consider briefly the evidence upon which each conclusion was based.

Sinclair's contention that liver fluke anaemia arises mainly from a failure of erythropoiesis was based largely on the results of personal studies in which the haematological and radioisotopic indices of experimentally infected sheep were compared with those of animals subjected to phlebotomy[3, 4]. These experiments revealed that sheep from which 60 ml of blood were removed daily developed less severe anaemia than animals harbouring 500 adult flukes, whereas those subjected to a daily blood loss of 130 ml, not only became more anaemic, but also showed a more marked erythropoietic response, as measured by rates of plasma ^{59}Fe clearance and red cell ^{59}Fe utilisation, than animals infected with about 100 adult Fasciola. Assuming that each fluke ingests about 0.2 ml of blood per day[5], then it can be calculated that the infected sheep were losing respectively about 100 ml and 20 ml of whole blood per day. Clearly in neither study was comparison/

comparison of bled and infected animals valid - indeed in many respects the differences recorded were only to be expected, a point later conceded by the author following more detailed radioisotopic investigations[6, 7].

Dargie's conclusion was reached largely on the basis of data reported from a number of laboratories between 1968 and 1972 involving the use of radioisotopic methods. Perhaps the most significant of these was the demonstration by Holmes and colleagues[8], that red cells labelled with ^{51}Cr disappeared more rapidly from the circulation of fluke-infected sheep than from their worm-free counterparts. This finding, which was later confirmed by Sewell and co-workers[9], and by Sinclair[6], provided evidence that increased erythrocyte destruction was involved in the pathogenesis of the anaemia. However, as Holmes pointed out, the more rapid removal of labelled cells from the blood of the parasitised animals could have arisen from a variety of factors, e.g. increased elution of isotope from the cells, increased red cell breakdown by intra - and/or extravascular haemolysis, and increased loss of red cells into the gastrointestinal tract. Taking advantage of two important properties of ^{51}Cr-labelled cells, namely that elution or haemolysis is followed by the appearance of isotope in the urine, whereas loss of cells into the gut results in quantitative excretion of isotope in the faeces [10 - 12], Holmes and colleagues[8], demonstrated that the reduced lifespan of labelled cells in infected animals was in fact associated with the appearance of large amounts of isotope in the faeces, urinary output being normal. Further by comparing the radioactivity of daily faecal collections with that of the blood, the/

the authors obtained estimates both of the total amount of blood or its breakdown products which entered the gut during that period, and of the daily blood loss per fluke; although somewhat variable this latter figure was generally about 0.2 - 1.0 ml [9, 13]. From these results the authors concluded that the adult liver fluke, rather than causing anaemia by releasing haemolytic toxins, (a mechanism referred to in some veterinary textbooks [14, 15]), exerted its principal pathogenic effect on the erythron by sucking blood from the vessels in the biliary mucosa. This theory received further support from the results of two subsequent investigations in which ^{51}Cr-labelled erythrocytes were again used. The first of these demonstrated that removal of adult flukes by anthelmintic treatment brought about a rapid cessation of intestinal red cell leak and a gradual return of the animal's haematological indices to normal values[16], while the second showed that marked anaemia in sheep developed with the onset of blood loss around the 10th week after infection[17]; this is the time when the flukes become established in the host's bile ducts[18].

The significance of the information provided by the ^{51}Cr technique was not merely that it implicated blood loss in the attendant anaemia; just as important was the demonstration that fluke-infected animals, after suffering early reductions in haematological values, were often able to maintain these (albeit at reduced levels) over prolonged periods of subsequent haemorrhage. In an indirect way this implied that such animals were in fact capable of producing increased numbers of red cells, but direct evidence necessitated measurements of red cell synthesis. Since this is only possible using/

using a label which is incorporated directly into haemoglobin, studies of iron metabolism using the radioisotope ^{59}Fe, which over the years had become a well-established and reliable tracer for quantifying erythropoiesis in man [19 - 22], were initiated by a number of workers [6, 13, 23, 24]. The results of experiments utilising this isotope revealed two important disturbances in the circulating iron exchange of animals suffering from chronic fascioliasis. First, the amount of iron carried via the plasma to the bone marrow for haemoglobin synthesis (the plasma iron turnover rate) was elevated and second, the rate of iron incorporation into new red cells was accelerated. These findings taken together with the ^{51}Cr results outlined earlier, led Dargie[2], to conclude that fluke-infected animals are often able to counteract the haemorrhage to which they are being concurrently subjected.

There now seems to be little or no disagreement on the basic validity of the "blood loss" theory, but the wide variation in the anaemia exhibited by infected animals suggest that it may not be the whole story. For example, the type of anaemia experienced by infected sheep has been reported to range from normocytic, normochromic[25], through macrocytic, normochromic[26], to macrocytic, hypochromic[27]. Many of these differences are probably attributable to variations in fluke burden and length of infection since the extent of the enteric blood loss is closely correlated to the number of parasites present[9, 13], but the fact that in Australia fatal fascioliasis in sheep occurs from infections of 125 flukes[28] while in Ireland levels greater than 250 flukes appear necessary[26], it is probably that the pathogenesis of this disease is not solely determined by fluke burden. While other factors/

factors such as the host's age, weight and possibly even breed may underlie these discrepancies, perhaps the most obvious variable is its nutritional status.

The relationship between nutrition and liver fluke anaemia was first implied by Reid and colleagues [29] who showed that when naturally infected cattle were brought indoors and fed hay supplemented with concentrates, their haematological indices improved. Likewise, Boray [30] reported lower PCV values in fluke infected sheep maintained solely on wheaten chaff, than in those fed a diet of 50% crushed wheat and 50% lucerne chaff. However, undoubtedly the most clear-cut demonstration to date of the considerable influence exerted by host diet on the development of this anaemia was given by the results of the previous chapter in this thesis, showing that the haematological indices of sheep restricted to hay deteriorated earlier and more rapidly than those of similarly infected animals provided with a high protein supplement. Unfortunately, these results did not in themselves give any indication as to how such differences arose although two possible mechanisms were suggested. Firstly, that as a result of their more rapidly developing parasite populations the poorer fed sheep experienced earlier, and at any given stage, more severe intrahepatic and biliary haemorrhage, and secondly, that relative to their protein-supplemented counterparts, the sheep restricted to hay suffered some impairment of erythropoiesis.

To establish the relative importance of these factors, it is necessary to have more precise and extensive comparative information on the functional state of the erythron as a whole under the different dietary/

dietary regimes, such as total red cell and blood volumes, rates of red cell breakdown and replacement in the animals concerned. This type of dynamic information can be obtained only by radioisotopic tracer techniques. Investigations using ^{125}I-albumin to measure plasma volume and hence red cell and blood volumes using the haematocrit, and ^{59}Fe to monitor haemorrhage via the gut and erythropoiesis were therefore carried out in the animals described earlier in an attempt to examine further the role of host diet in the development and pathogenesis of liver fluke anaemia.

MATERIALS AND METHODS

Experimental Animals and Design

Descriptions of the sheep used, the diets fed, and the basic designs of the two experiments performed were given in the previous chapter. Briefly, in the first experiment two groups each of 8 sheep were fed low (hay only) or high protein (hay plus concentrates) diets for 6 weeks; 5 members of each group were then each infected with 1,000 F. hepatica metacercariae, the remainder acting as pair-fed controls to 3 of the infected animals. Losses of blood and red cells into the gastrointestinal tract and of iron in the faeces were monitored continuously while blood volumes and plasma iron turnover rates were determined at intervals following intravenous injections of radioiodinated albumin and ^{59}ferric citrate.

The second experiment, in which no measurements were made of plasma iron turnover, involved 12 sheep, half of which were individually infected with 600 F. hepatica metacercariae and paired by weight with a worm-free partner. Paired feeding was continued on high protein diets for 16 weeks and subsequently on a low protein diet for 4 weeks.

The sheep were confined in standard metabolism cages throughout, and in addition to water which was available ad lib., 10 ml 0.75% KI was given orally each day to ensure rapid excretion of ^{125}I from degraded labelled albumin. Food consumption and the total output of faeces were recorded daily, and the sheep were weighed and blood and serum samples collected twice weekly for the determination of packed/

packed cell volume, haemoglobin concentration and serum iron concentration.

Diets

The basic composition of the diets used in these experiments was described earlier, but briefly the crude protein content of the hay varied between 4% and 9% while that of the concentrates was about 14% during Experiment 1 and the first 16 weeks of Experiment 2, and 8% during the subsequent 4 weeks. The iron content of individual batches of hay was very variable (160 - 660 mg/kg dry matter), but the compound rations which in all cases contained about 3.5 mg ferrous sulphate/kg were more uniform (300 - 370 mg/kg dry matter).

Haematological and Biochemical Analyses

Packed cell volumes (PCV), haemoglobin concentrations (Hb) and serum iron concentrations were measured by the techniques outlined previously.

Labelling, Injection and Sampling Procedures

Solutions of ^{125}I-albumin and ^{59}Fe citrate were prepared as described previously and injected via a jugular catheter before and at intervals after infection. At each injection individual sheep received approximately 500 µCi ^{125}I and 50 µCi ^{59}Fe (250 µCi in Experiment 2); standards were prepared by accurate dilution of measured volumes of each injected solution. For measurements of plasma iron turnover, blood samples were collected 15, 30, 60, 90, 120 and 180 minutes after injection and the plasma analysed for radioactivity. The 15 minute post-injection sample was also used for the determination of plasma volume. Blood and faecal samples were collected daily for radioassay.

Radioactivity Measurements

Radioactivity determinations on blood, plasma and faeces were carried out as described previously, the ^{125}I count rates of plasma samples collected 15 minutes after injections being corrected for any ^{59}Fe activity by γ-ray spectrometry and analysis of an ^{59}Fe standard solution at both ^{125}I and ^{59}Fe settings. Corrections for radioactive decay were also based on the activity of such standards.

Calculations and Expression of Results

Plasma, Red Cell and Blood Volumes

Plasma volumes (Vp) were estimated following each injection of ^{125}I-labelled albumin by dividing the total injected radioactivity by the activity of the plasma sample collected 15 minutes after injection.

Blood volumes (BV) were calculated from Vp and the PCV of the 15 minute blood sample and circulating red cell volumes (RCV) as the difference between BV and Vp. For comparative purposes all volumes were related to body weight. It should be stressed that while measurements based on dilution of labelled albumin provide a reliable estimate of Vp, differences inherent between venous and whole body haematocrits lead to variable overestimation of BV and hence RCV; nevertheless, the values obtained were considered sufficiently accurate for the studies envisaged.

Gastrointestinal Blood and Red Cell Losses

The ideal label for quantifying gastrointestinal haemorrhage should possess the dual property of remaining firmly attached to the red cell during its lifespan in the circulation, and most important of/

of all, should not be reabsorbed in the event of the red cell passing into the gut. The isotope which has come to be the generally accepted tag for the erythrocytes of man and a number of other animals is ^{51}Cr - basically because in these species it adequately fulfils the latter condition [10, 12]. However, in sheep, ^{51}Cr has been found to have at least one major drawback, i.e. it is rapidly eluted from labelled cells [31, 32], and this, together with its short physical half-life (27 days) and low energy emission render it technically inconvenient for long-term studies of the type described here. Moreover, it appears that some reabsorption of label can occur when tagged cells are given orally or directly into the abomasum[33], and hence red cell losses into the gut may be underestimated.

The alternative is to label the animal's erythrocytes in vivo with ^{59}Fe. This isotope with its long half-life (45 days) and readily detected γ photons (1.1 and 1.3 MeV), has the additional advantage over ^{51}Cr of remaining firmly attached to the red cell throughout its lifespan; together these factors make long-term investigations possible. The major disadvantage of ^{59}Fe is that once passed into the gut it may be absorbed and reutilised for red cell synthesis, and hence like ^{51}Cr (but potentially more so since iron is a natural metabolite), may underestimate the severity of any haemorrhage. It is important to bear in mind however, that faecal iron losses are normally minimal (1 - 2 mg per day) and arise mostly from desquamation of intestinal iron-containing cells [34, 35]. By contrast, in situations where significant haemorrhage occurs into the gut, the major form of iron reaching the lumen is haemoglobin.

The/

The question of whether ^{59}Fe can reasonably be used to quantify such haemorrhage therefore depends upon the animal's capacity to reutilise haem-iron. While there is certainly good evidence that carnivorous and omnivorous mammals (e.g. dog, rat and man) possess a mucosal haem-splitting enzyme - either xanthine oxidase[36] or microsomal haem oxygenase [37] - no such evidence is available for ruminants. Indeed, although Holmes and Maclean [38] reported extensive reabsorption of haemoglobin iron in one fluke-infected animal, most studies suggest that sheep, even when losing large amounts of blood into the gut, are unable to reutilise significant amounts of the constituent haemoglobin [23, 39 - 42]. It seems, therefore, that ^{59}Fe-labelled erythrocytes provide a useful tool for making reasonably quantitative measurements of gastrointestinal blood loss in sheep.

In the present experiments these were expressed as "faecal clearances" of whole blood and of red cells, obtained by dividing the total ^{59}Fe activity of each daily faecal collection by the activity per ml of whole blood and of red cells respectively. Such figures represent the amount of these constituents which would have to pass into the gastrointestinal tract each day in order to account for the radioactivity in the faeces.

Faecal Losses of Haemoglobin Iron

Each g of haemoglobin contains 3.34 mg iron, and, therefore, the amount of iron excreted in the faeces each day as a result of the haemorrhage may be calculated from the ^{59}Fe whole blood "clearance" and the blood haemoglobin concentration at that time using the formula [43]:

$$\text{Faecal iron excretion (mg/day)} = \frac{\text{Hb (g\%)} \times 3.34 \times {}^{59}\text{Fe "clearance" (ml)}}{100}$$

Plasma Iron Turnover Rates

Although only a very small part of the animal's total body iron (about 0.1%), plasma iron occupies a central role in iron metabolism since it represents the sole means by which this essential metal can be transported from one part of the body to another; this is accomplished by means of its attachment to the plasma protein transferrin. Since most of the functional iron in the body is present as haemoglobin which is constantly being broken down and re-synthesised, it follows that by far the major portion of the iron leaving the plasma at any time is being carried to the bone marrow for synthesis of this pigment. With the ^{59}Fe tracer techniques introduced by Huff [19] and Bothwell [21] and their colleagues it is possible to obtain quantitative data on the amount of iron being turned over through the plasma.

These involve labelling the plasma transferrin with ^{59}Fe (either by incubating ^{59}ferric citrate with fresh plasma prior to administration, or more simply by injecting the isotope directly), and measuring its rate of clearance over a period of 3 hours. During this interval, the decrease in plasma activity with time usually follows a single exponential curve when plotted on a semi-logarithmic scale and which on integration can be expressed as:

$$k = \frac{0.693}{t_{\frac{1}{2}}\ (min)}$$

where k represents the fraction of iron in the plasma removed per unit time, 0.693 the natural logarithm of 2 and $t_{\frac{1}{2}}$ the time taken for the plasma radioactivity to fall by 50%.

With/

With a knowledge of the serum iron concentration and plasma volume (Vp), the plasma iron turnover rate (PITR) may be calculated from the equation:

$$\text{PITR (mg/day)} = \frac{\text{Serum Iron (mg/ml)} \times \text{Vp (ml)} \times 0.693 \times 1440}{t_{\frac{1}{2}} \text{ (min)}}$$

where 1440 = number of minutes in the day. To allow comparison between individuals this rate was related to body weight.

A simpler standard of reference relates plasma iron turnover to 100 ml whole blood. This allows better comparison between individuals since it is to be expected that a constant amount of iron would be required to provide the iron for the red cells in 100 ml blood. Rates expressed in this way were calculated by substituting the relevant data into the following equation:

$$\text{PITR (mg/day/100 ml blood)} = \frac{\text{Serum Iron (mg\%)} \times 0.693 \times 1440}{t_{\frac{1}{2}} \text{ (min)}} \times \frac{100 - \text{PCV}}{100}$$

It must be stressed that plasma iron turnover rates do not quantify absolutely the amount of iron utilised for haemoglobin synthesis - they are in fact an overestimate because of two factors which together increase the plasma ^{59}Fe clearance rate beyond that compatible with haemoglobin formation. The first is storage iron, i.e. a proportion of the iron leaving the plasma is transported not to the bone marrow, but to the iron stores of liver, spleen, gut, etc. Secondly, of the iron carried to the marrow, approximately 25% becomes reversibly fixed to a labile erythropoietic iron pool present at or within the membrane of the developing red cells, and is subsequently fed back into the plasma. Consequently, the results quoted in the text should not be considered quantitative but rather as approximate indices of erythropoiesis. A more detailed treatise of the plasma iron turnover rate and its interpretation is given by Pollycove and Mortimer[22].

RESULTS

EXPERIMENT 1

Two groups of Scottish Blackface wethers were fed rations consisting either of chopped hay or chopped hay supplemented with a high protein compound diet. Six weeks later 5 animals in each group were individually infected with 1,000 F.hepatica metacercariae, the remainder acting as pair-fed controls to 3 of the infected sheep. Blood volumes and plasma iron turnover rates were measured periodically and haemorrhage into the gut via the bile daily over the following 4 - 5 months. For ease of presentation only the average values recorded for the pair-fed sheep are included in the illustrations, individual data for all sheep being presented in Appendix 2.

The changes in PCV and crude protein intake recorded in these animals have been described in detail in the preceeding section but, in view of their relevance to the data presented here, the mean values are illustrated again in Fig. 1 for convenience.

Blood Volumes

The results of the plasma volume measurements made using ^{125}I-albumin, and of the estimates of red cell and blood volumes based on venous haematocrit are illustrated in Fig. 2a and b.

When the sheep were placed on their respective diets, the size of each compartment was very similar in all animals. On repeating the measurements 6 weeks later (i.e. at the time of infection) it was found that plasma volumes had increased and red cell volumes decreased under each plane of nutrition. Although both compartments were 10 - 15% larger in the supplemented sheep ($p < 0.01 - 0.001$), these/

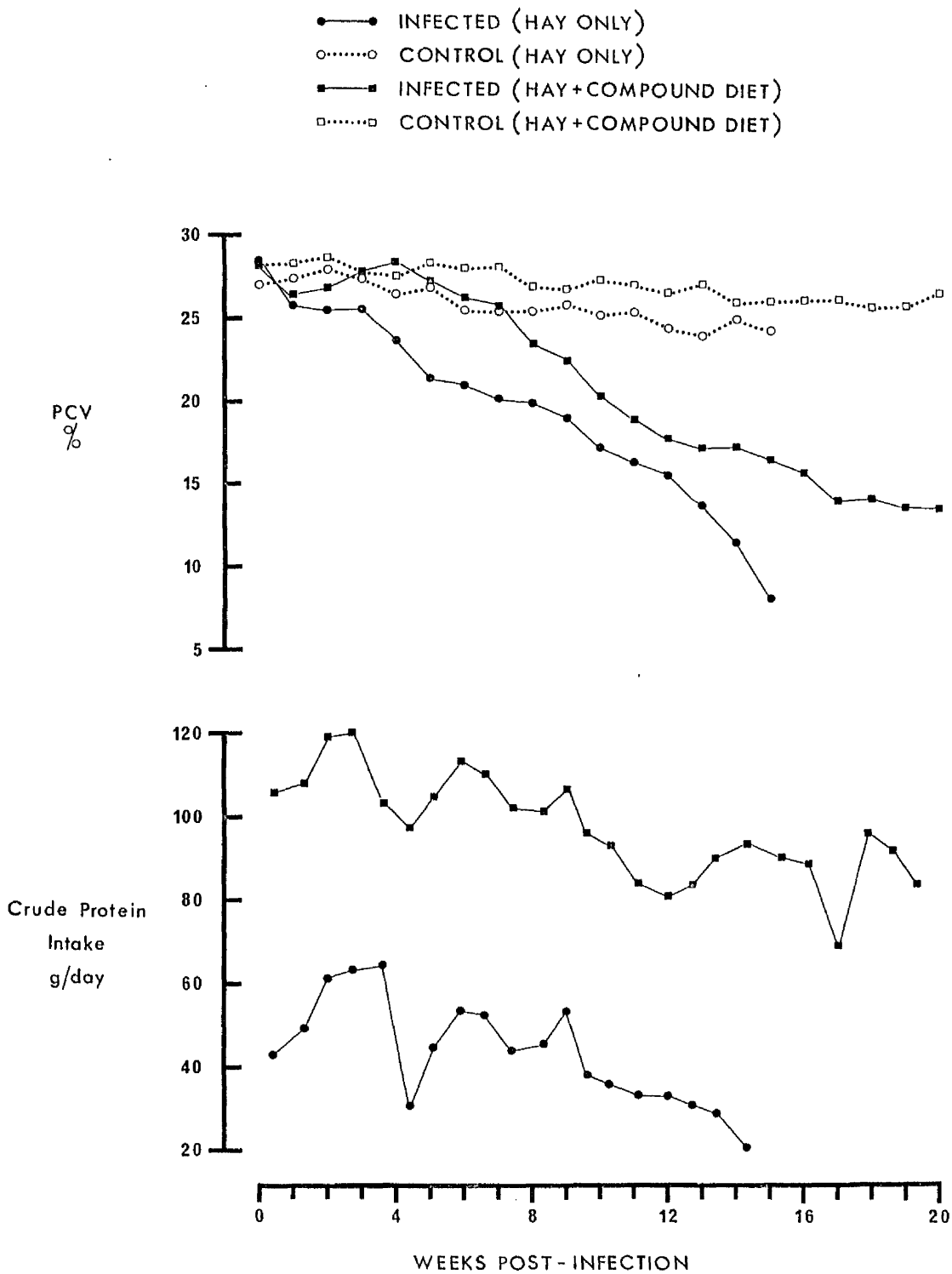


Fig. 1 : PCV Values and Crude Protein Intakes of Fluke-Infected and Control Sheep.

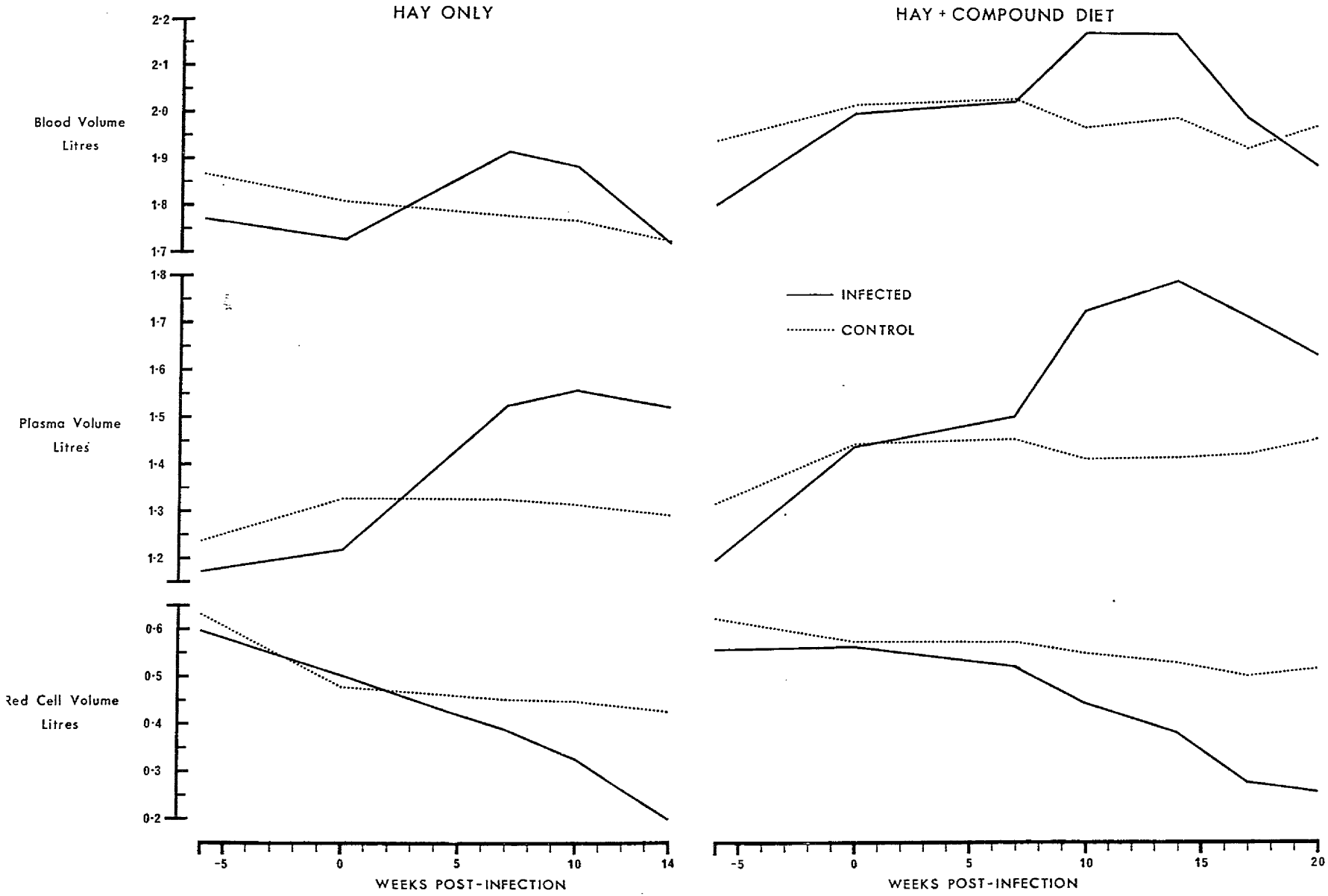


Fig. 2a : Effect of Diet on the Blood Volumes of Fluke-Infected and Control Sheep.

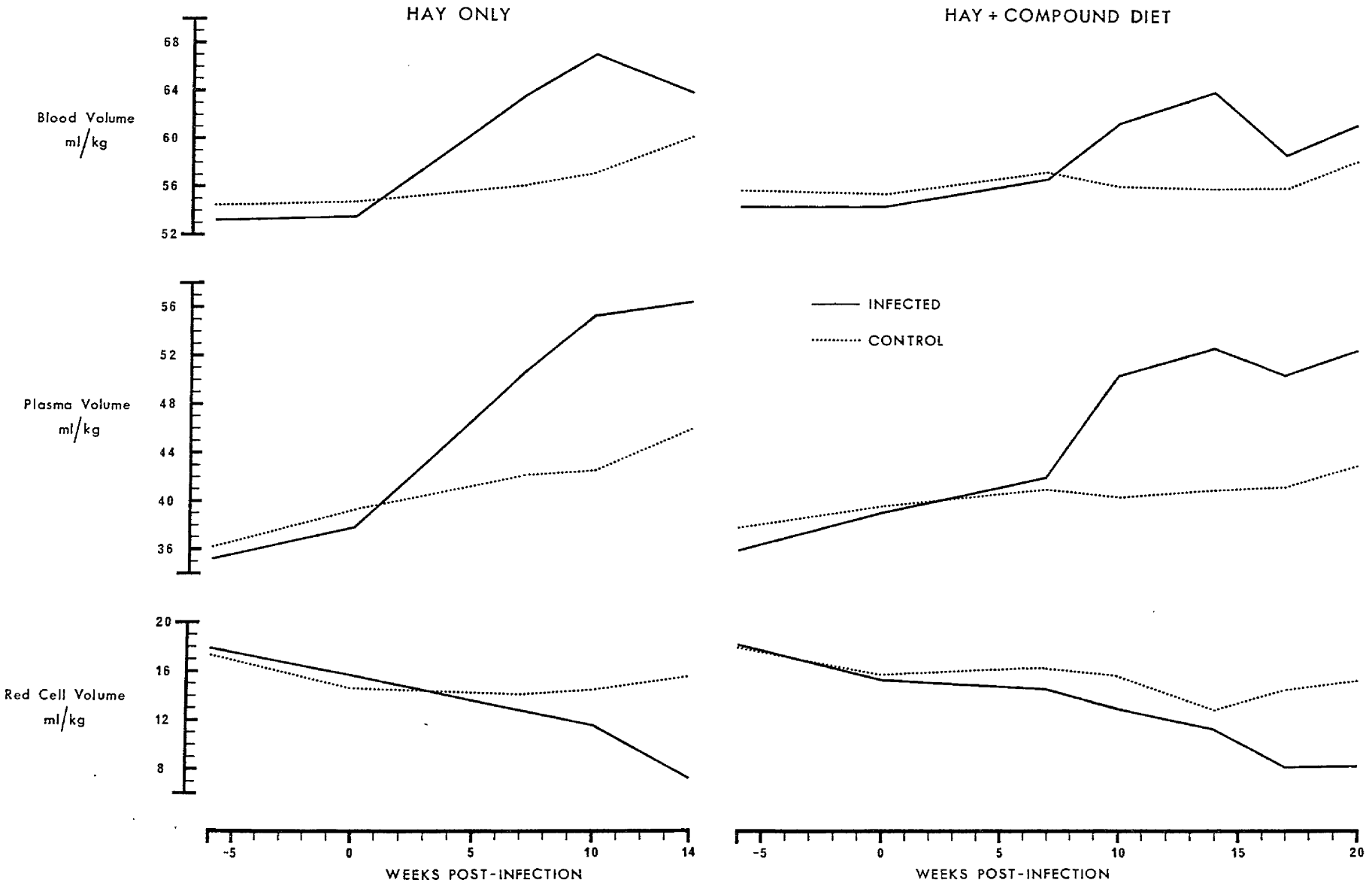


Fig. 2b : Effect of Diet on the Blood Volumes (related to body weight) of Fluke-Infected and Control Sheep.

these differences were not significant when related to body weight. In other words one of the major factors responsible for the changes and group differences recorded at this time was the superior growth rate of the better-fed sheep. The other was the slight anaemia and hence reduction in red cell mass arising from blood sampling; this also contributed to the slight expansion in plasma volume. The overall result was that the total blood volume of the protein-supplemented sheep increased in proportion to growth, and remained unchanged in those given only hay.

As the disease developed, sheep on each diet experienced further and more substantial expansions in plasma and reductions in red cell volumes, and while these changes were ultimately of the same severity, their onset was earlier in animals restricted to hay. Moreover, whereas plasma volumes increased sharply over a relatively short period, i.e. during the first 7 weeks in the low protein group and between the 7th and 10th weeks in the supplemented animals, and thereafter remained elevated, red cell volumes continued to deteriorate as the experiment progressed. As a result, apart from the periods of marked plasma expansion when increases in plasma volume were about three times greater than the estimated red cell deficits, and total blood volumes also increased, animals on each diet maintained high but relatively constant blood volumes until necropsy. Figures for the controls generally remained steady except that the plasma volumes of the poorer fed sheep increased slightly relative to bodyweight between the 10th and 14th weeks.

Gastrointestinal Blood and Red Cell Losses

During the experiment the sheep received periodic injections

of/

of ^{59}Fe as ferric citrate, and each day the activities due to this isotope in the blood and red cells were divided into the total faecal activity to monitor the development of haemorrhage into the gut via the bile. The results of these measurements which are expressed in Fig. 3 as faecal "clearances" of whole blood and red cells respectively show that while both groups of infected sheep experienced progressively more severe haemorrhage by this route from about the 8th week, the rate of blood loss, at least over the following 6 weeks, was faster in the animals restricted to hay. Between the 7th and 14th weeks the poorer fed sheep lost about 3,500 ml of blood and those on the better diet 2,700 ml, a difference of about 32%. However, since the former were in any case more anaemic, total red cell losses differed by only 40 ml or 8% (510 ml and 470 ml respectively), and were probably virtually identical if losses due to blood sampling are taken into consideration. When related to the attendant changes in red cell volume, these figures indicate that all animals, but particularly those receiving the better diet, experienced total red cell losses far in excess of that reflected by their circulating red cell deficits (i.e. 190 ml and 135 ml respectively in the low and high protein groups).. In an indirect way this implies that erythropoiesis was accelerated in both groups, but more so in the latter.

Of even greater significance are the data obtained between the 14th and 20th weeks showing that on average the supplemented sheep lost a further 7,250 ml of blood prior to their eventual necropsy, representing about 1,050 ml red cells. Since these losses were accompanied by a drop in red cell volume of only 140 ml, it would appear/

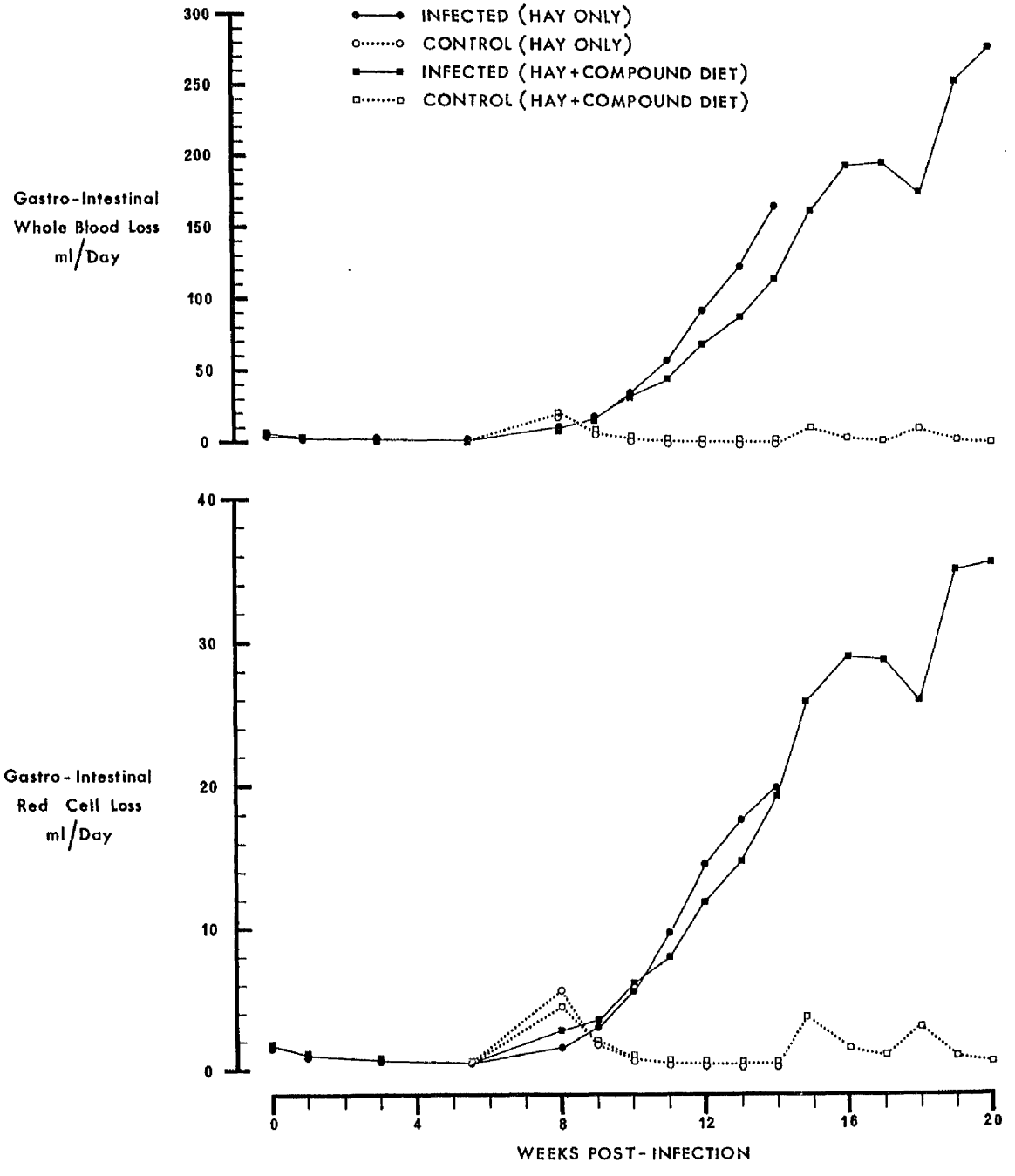


Fig. 3 : Effect of Diet on the Gastro-Intestinal Blood and Red Cell Loss in Fluke-Infected and Control Sheep.

appear that by this stage of the disease red cell synthesis was substantially higher than earlier.

At 14 weeks, blood loss per fluke was 30% higher in the poorer fed sheep (0.32 ± 0.05 ml/day compared with 0.22 ± 0.09 ml/day), but individual variation precluded significance and red cell losses were almost identical (0.04 ml/day). However, in the supplemented animals, blood and red cell losses per fluke (0.55 ± 0.06 ml/day and 0.07 ± 0.001 ml/day respectively) were both significantly higher at 20 weeks than at 14 weeks ($p < 0.02$).

Faecal Haemoglobin Iron Losses and Iron Intake

One of the major consequences of biliary haemorrhage is that excessive quantities of iron pass into the gut as haemoglobin; if not reabsorbed this iron is lost from the body in the faeces. Such losses may be estimated from the ^{59}Fe faecal "clearances" of blood and the haemoglobin concentration. The results presented in Fig. 4 show that faecal iron losses increased progressively and at a similar rate in both infected groups between the 8th and 14th weeks. Subsequently, further and even more dramatic losses were experienced by the group receiving concentrates with the result that by the termination of the experiment, the total amount of iron lost by these sheep was about $2\frac{1}{2}$ times greater than in the sheep restricted to hay (about 1,800 mg compared with 750 mg).

Also given in Fig. 4 are the data on iron intake. Apart from a sharp drop between the 3rd and 5th weeks, which was caused by feeding hay with a particularly low iron content, the iron intake of both infected groups generally ranged between 250 mg and 350 mg/day during/

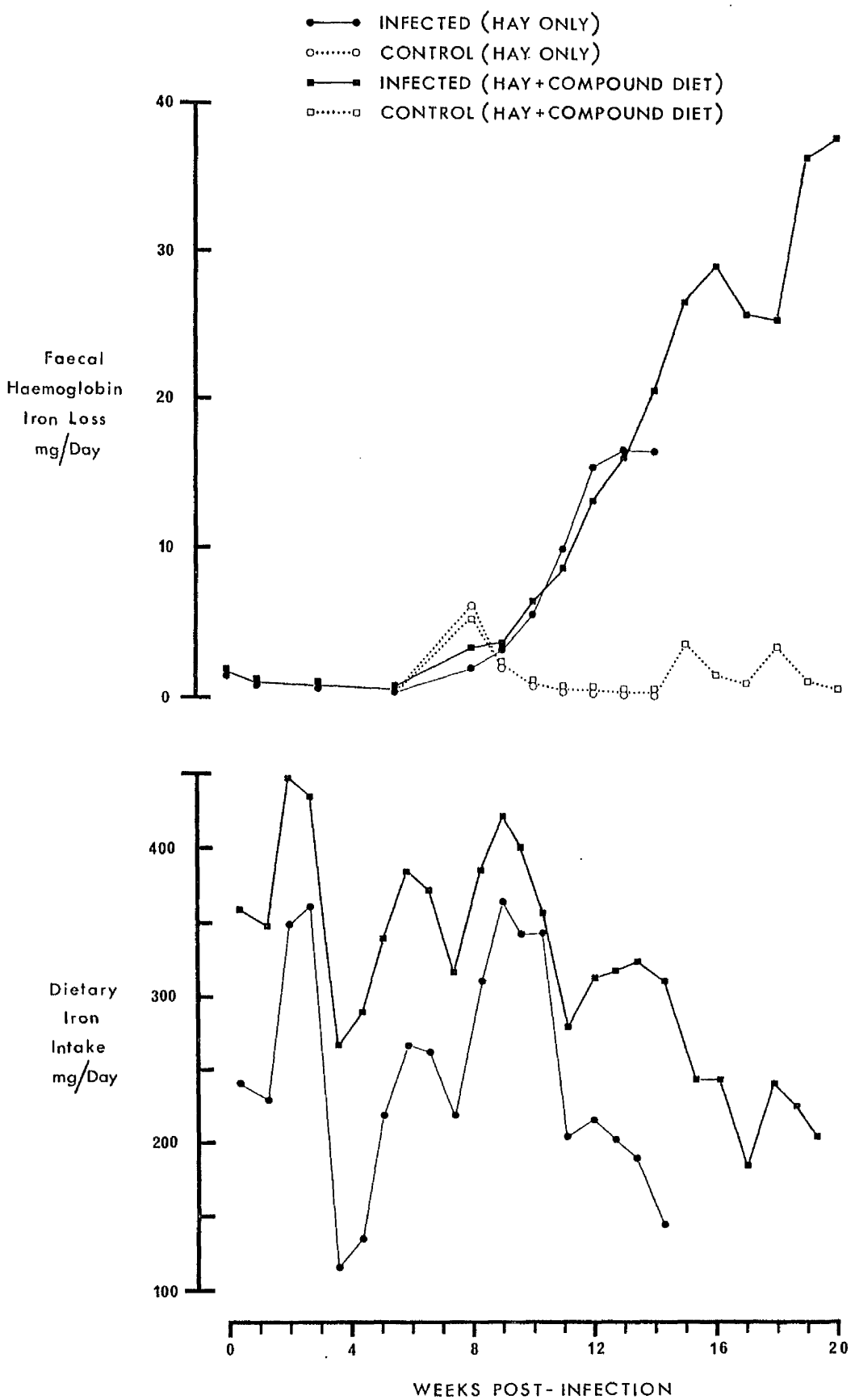


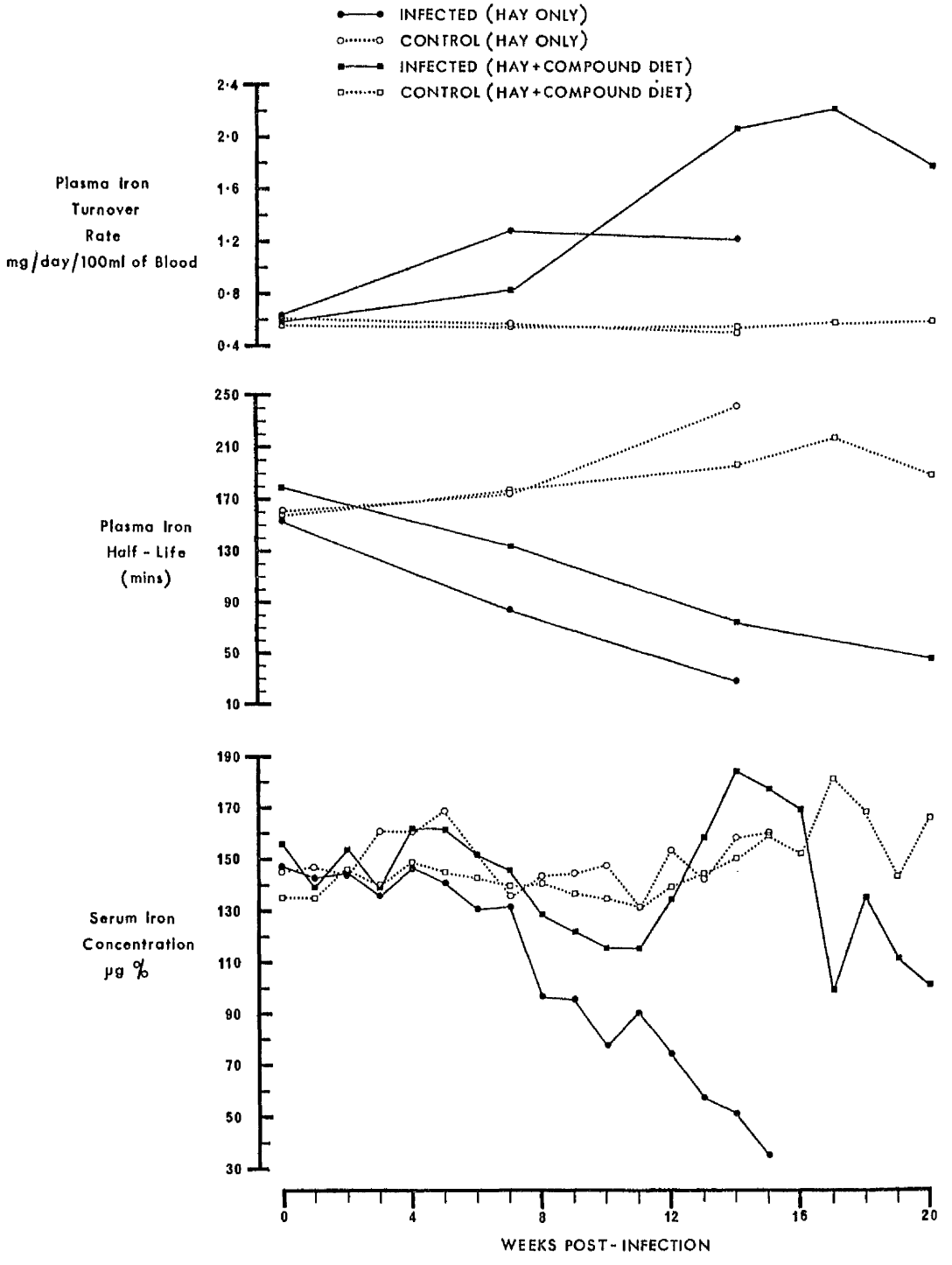
Fig. 4 : Faecal Haemoglobin Iron Loss and Iron Intakes of Fluke-Infected and Control Sheep.

during the first 10 weeks and was 40% lower in the animals restricted to hay. Thereafter, and largely as a result of inappetence, iron intake fell in all animals, but more rapidly in the poorer fed group whose consumption by the 15th week was reduced to about 120 mg/day compared with 220 mg/day in the sheep receiving concentrates; this level was maintained subsequently in the latter group, but significantly the average intake of both groups was comparable during the 5 weeks before necropsy.

Plasma Iron and its Turnover

A further consequence of excessive iron loss is that by lowering the level of iron supply to the bone marrow erythropoiesis is ultimately suppressed. To examine iron supply in the experimental animals and its relationship to erythropoiesis, serum iron concentrations were measured weekly and total plasma iron and plasma iron turnover at intervals during the investigation. The results of some of these measurements are illustrated in Fig. 5. In all animals both the concentration and total amount of iron in the plasma were well maintained during the initial 7 weeks (i.e. within the range 140 - 170 µg % and 1.7 - 2.0 mg respectively), and control values deviated little from these figures throughout the remainder of the investigation. On the other hand both groups of infected sheep experienced gradual reductions in plasma iron concentration between the 7th and 11th weeks, but since the plasma volumes of the better fed animals increased at the same time, only in those restricted to hay was this accompanied by a drop in total plasma iron (to 1.4 mg). The downward trend continued in the poorer fed sheep and by the 14th week the concentration

and/



Fig, 5 : Effect of Diet on the Ferrokinetic Indices of Fluke-Infected and Control Sheep.

and total amount of iron in the plasma of these animals had deteriorated to 40 µg % and 0.7 mg respectively. By contrast, iron concentrations of the supplemented group improved transiently (to 175 µg % on week 15) only to decline again during the last 5 weeks (to 100 µg on week 20); as a result total plasma iron increased (to about 3 mg) and then fell to a value comparable to that recorded before infection.

Equally dramatic changes were noted in the rates of ^{59}Fe clearance from the plasma, which increased in the infected sheep and decreased in the controls as the experiment progressed. Both features were initially more marked in the poorer fed sheep but whereas all infected sheep ultimately experienced comparable reductions in $t_{\frac{1}{2}}$ values (about 75% between infection and necropsy), the clearance of ^{59}Fe was always slower in the control sheep restricted to hay, $t_{\frac{1}{2}}$'s lengthening by 50% during the first 14 weeks compared with the 25% increase recorded in the supplemented group over 20 weeks.

The basic point which emerges from the measurements of plasma iron turnover is that although this increased to a greater extent in the hay only group during the first 7 weeks of infection (i.e. by 100% compared with 40%), these animals were unable to attain the dramatic and sustained increase noted subsequently in their better fed counterparts. By the 14th week, iron turnover through the plasma of this latter group was 350% higher than before infection and about double that of the group fed only hay, increased further between the 14th and 17th weeks, and only subsequently showed a slight tendency to decline. The changes observed in the control sheep were much/

much less pronounced although values for the group restricted to hay fell by about 10% between the 7th and 14th weeks and by this stage were also 10% lower than those of the supplemented animals.

EXPERIMENT 2

Six Scottish Blackface wethers were each given 600 F. hepatica metacercariae, and paired to a worm-free control animal, whose feed intake was restricted to the same level as its infected partner. All sheep were maintained on a high protein ration (13 - 15% crude protein) during the first 16 weeks of infection and on a low protein diet (8% crude protein) for a period of 4 weeks thereafter. Blood volumes were measured at intervals during the study and the loss of blood into the gut estimated daily. In the interests of clarity only the mean values for infected and control groups are depicted in the figures but individual data are presented in Appendix 2.

A detailed description of the changes in PCV and crude protein intake recorded during this study have been presented previously but for convenience the values are illustrated again in Fig. 6.

Blood Volumes

The results of the blood volume measurements made before infection and again at monthly intervals thereafter are shown in Fig. 7. The total blood volumes of infected sheep were essentially the same as the controls throughout, increasing in parallel with body weight during the first 10 weeks and falling slightly thereafter. However, in association with the anaemia which developed from about the 6th week, red cell volumes of the infected sheep decreased and

plasma/

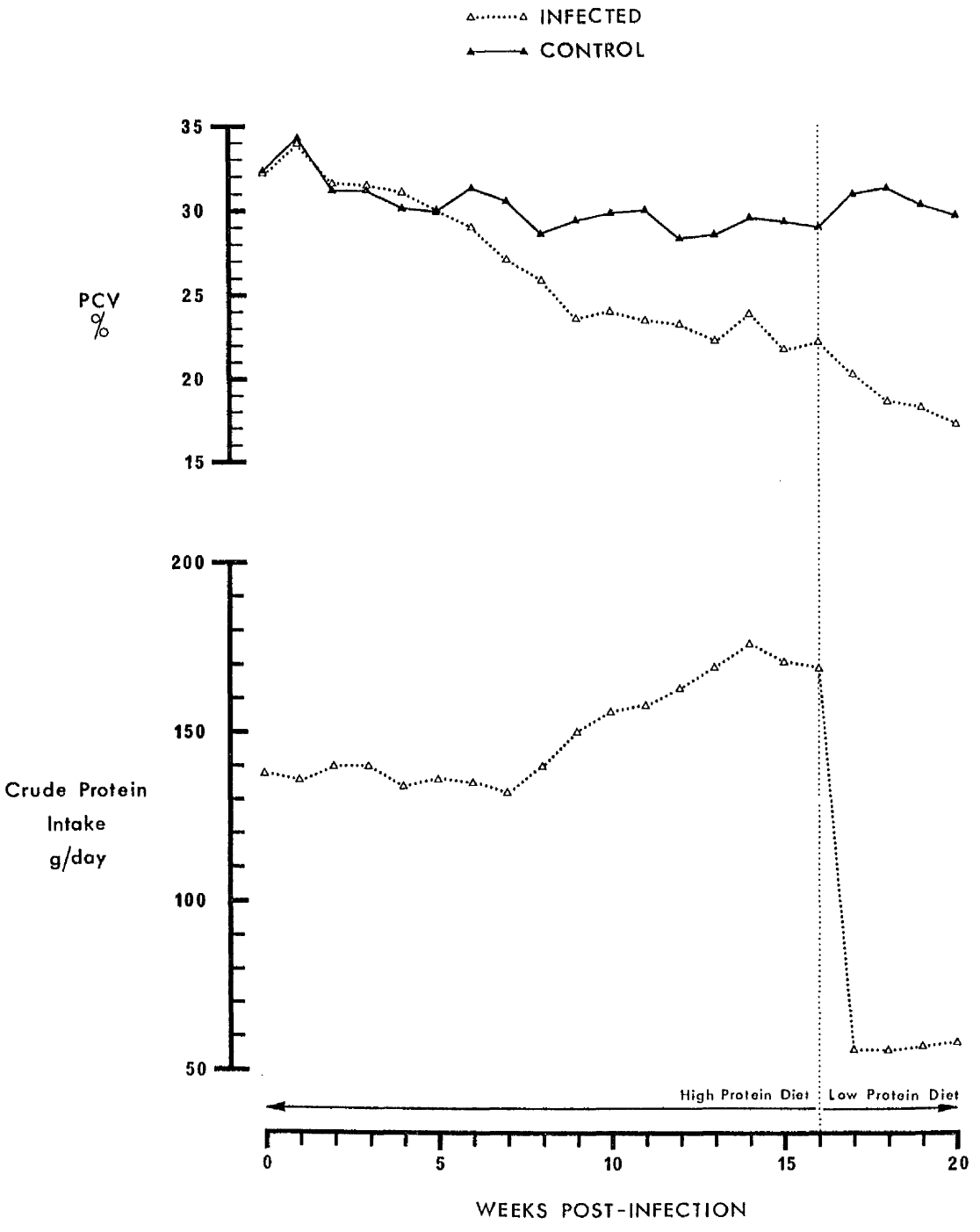


Fig. 6 : PCV Values and Crude Protein Intakes of Fluke-Infected and Control Sheep.

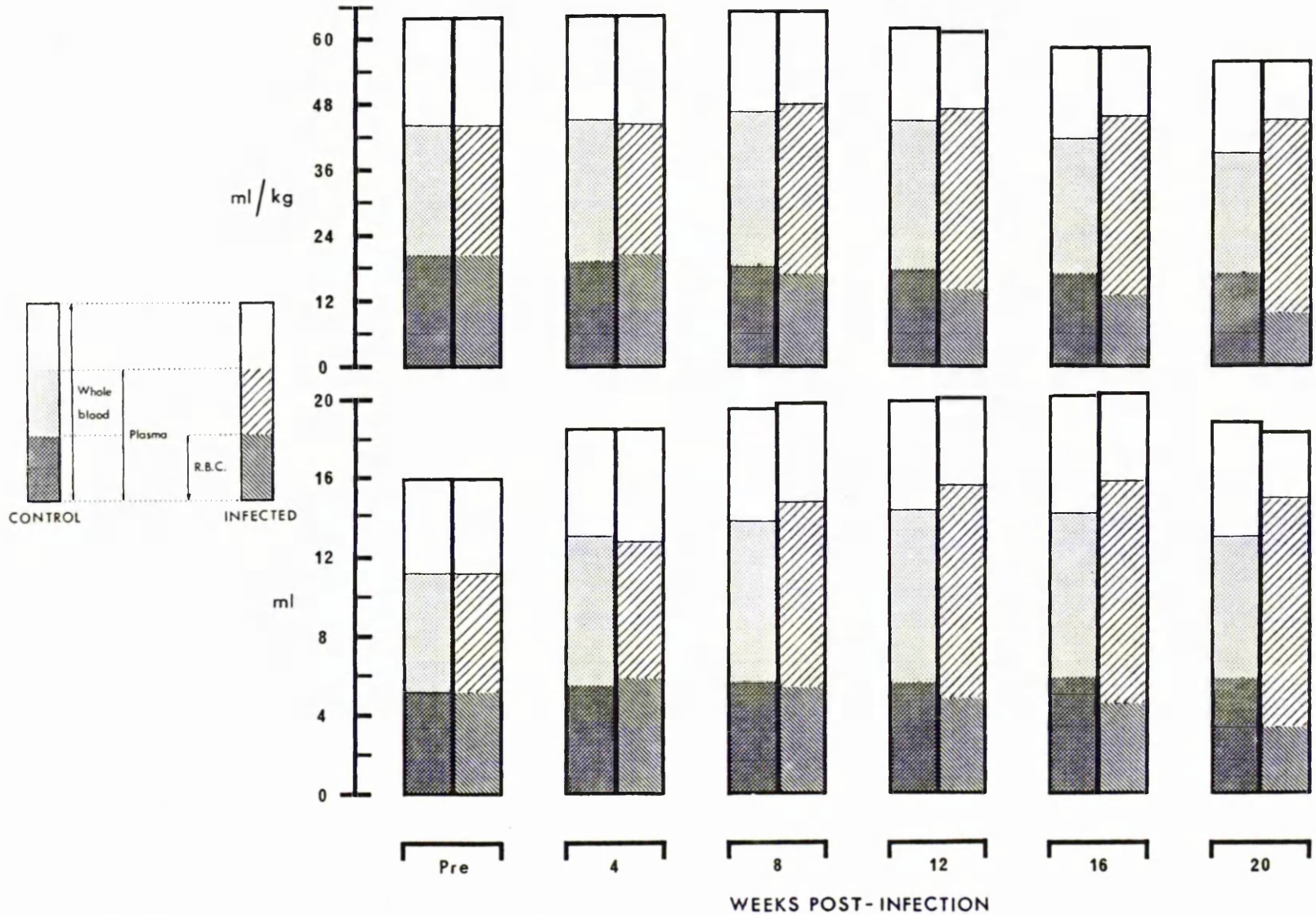


Fig. 7 : Effect of a Change of Diet on the Blood Volumes of Fluke-Infected and Control Sheep.

plasma volumes increased relative to the controls, both changes developing more rapidly following the switch to low protein feeding. This is particularly well illustrated by the figures for red cell volume which fell by 9% in the infected sheep during the 4 weeks immediately preceding the dietary change but by a further 25% over the same period thereafter. These changes were not observed in the controls which maintained their red cell mass throughout. Likewise, whereas the controls exhibited a steady decrease in plasma volume relative to weight, in the infected sheep this compartment remained high and by the 20th week was 16% greater than the corresponding control figure.

Gastrointestinal Haemorrhage, Faecal Iron Losses and Iron Intake

The pattern of blood loss exhibited by the infected sheep (Fig. 8) was broadly the same as before except that haemorrhage commenced 7 - 10 days earlier and attained maximum severity around the 14th week (100 ml/day). By this stage total blood losses (3 litres) were approximately the same as recorded earlier, but the accompanying losses of red cells and haemoglobin iron (700 ml and 800 mg respectively) were considerably greater because of the higher PCV's and haemoglobin levels of the sheep in this experiment. By the 16th week losses had risen to 4.5 litres blood, 1 litre red cells and 1,150 mg iron respectively, and following the dietary change the animals lost a further 3 litres blood, 600 ml red cells and 650 mg iron. Blood and red cell losses per fluke (0.58 $\pm$ 0.09 ml/day and 0.13 $\pm$ 0.02 ml/day respectively) were significantly higher at 14 weeks ($p < 0.02$) than at the corresponding stage of the earlier experiment, but remained at this level subsequently.

Iron/

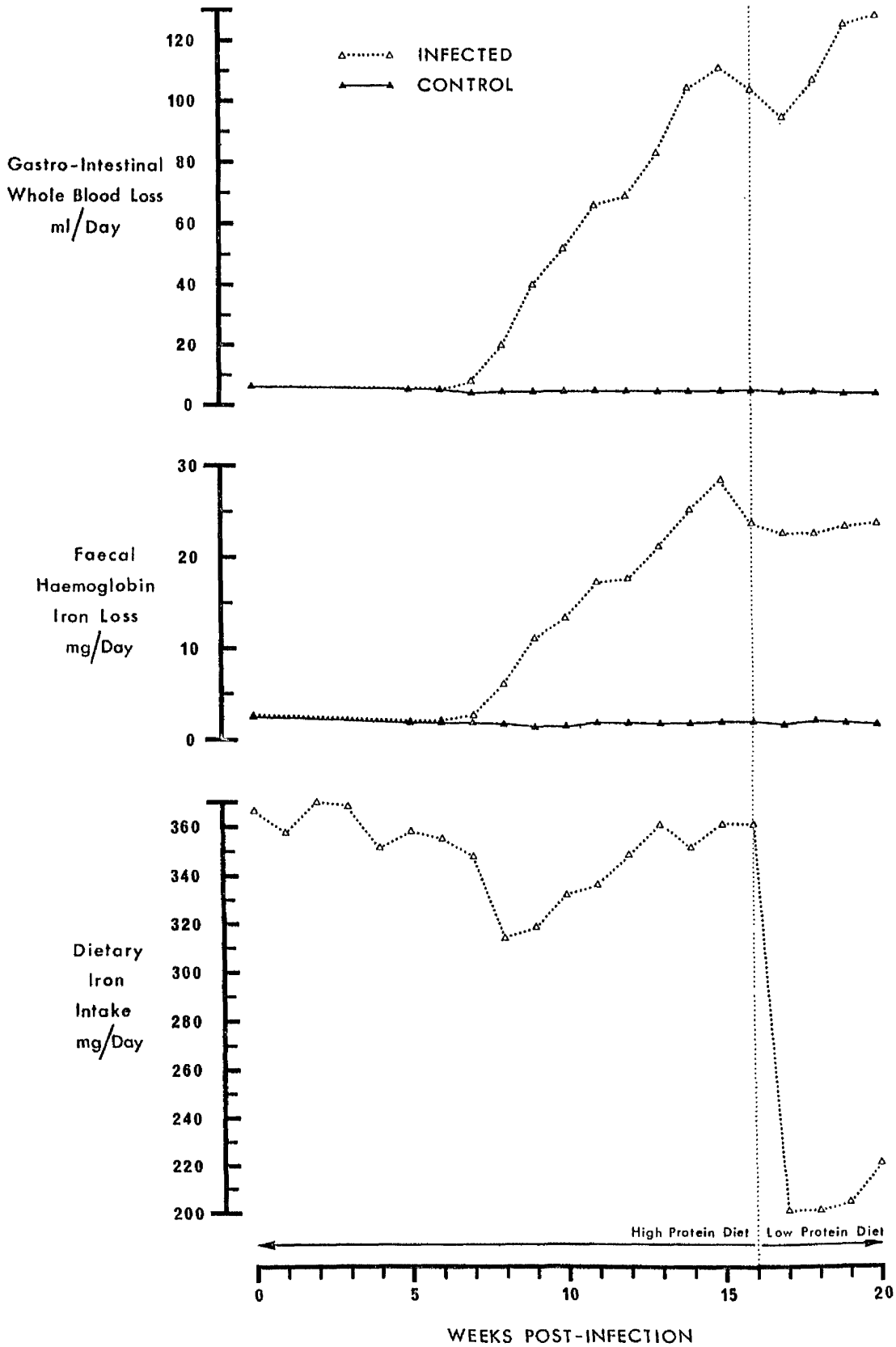


Fig. 8 : Gastro-Intestinal Blood and Haemoglobin Iron Losses, and Iron Intakes of Fluke-Infected and Control Sheep.

Iron intake during the period of high protein feeding ranged between 300 and 350 mg/day, but fell to 200 mg/day following the dietary change (Fig. 8).

Red Cell Synthesis

Erythropoiesis was not measured directly in this experiment but a useful insight into this aspect may be obtained by comparing red cell losses with the accompanying changes in PCV and circulating red cell volume. During the 4 weeks immediately preceding the dietary change, the PCV and red cell volumes of the infected sheep were well maintained despite red cell losses totalling 3 litres, but when superimposed on a lower plane of nutrition comparable losses caused a 20% drop in both values. This suggests that red cell synthesis was increased at both stages of the infection but to a much greater extent when the animals were receiving the better diet.

DISCUSSION

The results presented in the first chapter of this thesis demonstrated that the development of anaemia in sheep infected with F. hepatica depended on an interplay between the intensity of infection and the host's plane of nutrition. The work described in this section was an attempt to clarify the mechanisms underlying the haematological changes recorded earlier, and thereby the role of diet in the pathogenesis of this condition.

The involvement of haemorrhage in this disease has already been well documented [2, 8, 9, 23]. Therefore, in some respects the present findings showing that the most rapid deterioration in the haematological indices of infected sheep coincided closely with the loss of progressively more blood into the gut merely reinforce the view that the liver fluke exerts its principal pathogenic effect by sucking blood from the vessels of the biliary mucosa. Equally apparent however is that the "blood loss" theory, although basically sound, fails to explain several important features of the anaemic process. In particular, it fails to explain why some sheep experienced an earlier and more severe drop in PCV during fluke migration than others with a similar fluke burden but provided with more dietary protein and iron. But even more important, it does not adequately explain how the better fed sheep were able to maintain PCV's of 15% even after losing 9 litres of blood while their poorer-fed counterparts succumbed with PCV's of 9% following blood losses totalling only 3½ litres.

The anaemia associated with fluke migration has long been considered a consequence of intrahepatic thrombosis, and its severity

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a reflection of parasite burden, but since no method has yet been developed to quantify this haemorrhage its importance in relation to other possible aetiological factors is clearly impossible to assess. The situation is further complicated by the difficulty of defining the parasites' migratory period since the interval between the entry of the first and last flukes into the bile ducts is often quite protracted, especially in heavy infections which are invariably accompanied by delayed tissue migration and subsequent retardation of growth [28]. In the experiments reported here, the parasites' entry into and activities within the biliary system were monitored by measuring blood loss into the gut; in this way it was hoped to define more closely the effect of such variables as levels of infection and host nutrition on the parasite and host alike.

From the data obtained it is evident that not only was the period of tissue migration prolonged in animals with heavier infections, but the amount of blood lost per fluke was initially lower, indicative of a reduced metabolic activity and hence presumably an inferior growth rate. There is also a suggestion that the growth and feeding activities of individual parasites within a given population are relatively suppressed in animals maintained on a high plane of nutrition. This is based on two observations: namely that at 20 weeks the length of the flukes recovered from the sheep given concentrates (16.8 mm) was only marginally greater than that recorded around the 15th week of those restricted to hay (14.7 mm); and that between the 11th and 14th weeks the rate of blood loss was appreciably higher in the latter. At the same time it should be emphasised that both the/

the time at which the first flukes entered the bile ducts and the rate of blood loss during the subsequent 2 - 3 weeks were the same irrespective of diet, and that at no stage was the amount of blood lost by the better fed sheep less than that recorded during the previous week in their poorer fed counterparts. On balance, it would therefore appear that host nutrition had only a marginal effect on fluke migration and growth. This being the case it is difficult to ascribe the earlier development of anaemia in the poorer fed sheep to more severe intrahepatic haemorrhage, although the possibility cannot be discounted entirely.

There was however one basic difference between sheep fed low and high protein rations in their response to fluke migration, namely the earlier expansion of plasma volume in the former, i.e. during the first 7 weeks as compared with between the 7th and 10th weeks in the latter. Significantly it was during these periods that PCV's fell most dramatically in each group (from 27% to 20%) suggesting that haemodilution played an important part in initiating the anaemia presented by these more heavily infected animals. Whilst the aetiology of this previously unrecorded feature is unknown, the probable explanation lies either in the greatly increased serum globulin levels of these animals (see chapter 3)and/or the grossly deformed architecture of the fibrotic liver and its vasculature. The recent acrylic cast studies of Murray and Rushton [44], showing the development of occlusive lesions in the portal and hepatic veins and of thickened tortuous arteries in contaminated livers are clearly indicative of portal hypertension [45], the sequelae of which include abnormal water and salt retention [46]. One can therefore only assume that the more rapid onset of these disturbances/

disturbances in the poorer fed animals was a reflection of earlier liver dysfunction but whether this was attributable to different patterns of fluke migration or a failure to reconstruct damaged tissue requires further investigation.

However, if any other single fact emerged from these studies it was that sheep on a high plane of nutrition were much better able to withstand the biliary haemorrhage caused by their adult fluke populations than animals given rations containing less protein and iron. The obvious reason for this is that liver fluke anaemia is in part a nutritional problem, its severity at least as judged by PCV and red cell morphology being determined as much by the host's erythropoietic capacity as by the parasites' specific pathogenic effects. The principal benefit derived from the higher plane of nutrition, as demonstrated by the results of the first experiment and clearly implied by those of the second, is that it enables the erythropoietic system to respond in a much more positive and sustained manner to haemorrhagic stress. However, the relative importance of the many possible factors underlying the different responses observed are more difficult to assess and merits some discussion.

Protein restriction per se is a potent inhibitor of normal erythropoiesis [47, 48, 49] an effect mediated via reduced erythropoietin production [50] and depletion of the erythropoietin-sensitive stem cell pool [51]. It is, therefore, tempting to attribute the relatively poor response of the sheep on lower protein rations to an inadequate production of this hormone and hence supply of red cell protein precursors/

precursors to the marrow, but two considerations make this unlikely. Firstly, their control counterparts, which were restricted to the same range of protein intake (30 - 50 g crude protein/day) maintained their haematological indices at values comparable to those of the better-fed controls whose protein intake generally exceeded 100 g/day. Secondly, while it is known that protein-deprived animals utilise their protein pools sparingly in the physiological replacement of red cells when anaemic [47], red cell synthesis takes precedence in amino acid utilisation [52]. That a similar situation developed in the present studies is suggested by the rates of plasma iron clearance which fell progressively in the controls, but continued to accelerate in the infected sheep as protein intake dropped. Therefore, there are no real grounds for supposing that in the present studies the functional integrity of the bone marrow is in itself compromised by protein deficiency; the variation in response observed between individuals fed high and low protein diets was therefore presumably related to differences in the availability of some other building material(s) necessary for red cell replacement.

From the data presented, iron would appear to be the major limiting factor since, at least in the first experiment, the anaemia presented by the poorer fed animals was accompanied latterly by several criteria which although not diagnostic, were certainly indicative of iron deficiency: namely, hypochromia, as judged by MCHC, hypoferraemia, and despite a greatly accelerated plasma iron clearance, a relatively slow delivery of plasma iron to the marrow. Whilst the data presented in Fig. 4 leave little doubt that the primary cause of/

of this condition was excessive loss of haemoglobin iron in the faeces,it is equally apparent that since even greater losses were suffered by the supplemented sheep with little sign of impending deficiency, some additional factor(s) was involved. A similar conclusion can be drawn from the second experiment in which sheep on a high plane of nutrition appeared well able to counteract significant losses of iron, but when placed under nutritional stress the same losses were poorly tolerated.

In view of the marked hypercatabolism of plasma proteins which accompanied this disease[2, 53] and the causal relationship in man between protein restriction and hypotransferraemia [54] one possibility is that the different erythropoietic responses reflected variations in plasma iron binding capacity (TIBC) and hence transport efficiency. The present investigations provide no direct information on this point, but judging by the serum β-globulin levels, which include transferrin, this seems unlikely. In both experiments these increased sharply during the initial 10 weeks of infection, indicative of an elevated TIBC, and although falling subsequently in the first experiment, were neither abnormally low nor significantly different in the two infected groups at necropsy; in the second experiment, β-globulins of the infected sheep remained elevated following the switch to low protein feeding. These findings not only support earlier observations of an increased TIBC in fascioliasis [4, 55] but also the general concept of increased transferrin synthesis being part of the normal iron haemostasis in situations where iron delivery to the marrow is threatened, e.g. iron deficiency [56] and protein-losing gastroenteropathies of various/

various aetiology [57]. Since the requirements of the bone marrow for iron are normally met from the diet, and when this is insufficient by mobilisation of iron stores, the different erythropoietic capacities observed were therefore presumably related to variations in the amount and/or availabilities of this element from one or both of these sources.

Unfortunately, there is no information in the literature concerning the iron requirements of sheep or its availability from different feedstuffs and no attempt was made in these studies to measure iron stores directly. Consequently, it is difficult to construct a working hypothesis regarding the efficiency with which iron was handled by these animals. Nevertheless, it is unlikely that differences in the amount or availability of storage iron contributed substantially to the different erythropoietic responses observed. In the first place, all the animals were reared under identical conditions, and since their bodyweights were initially comparable, their iron stores were probably similar. Secondly, being relatively stable, and present in only small amounts (about 10 mg/kg [58]), storage iron rarely plays a dominant role in meeting iron requirements for erythropoiesis, and in these sheep would have provided little more than 300 mg, i.e. less than half that lost through haemorrhage by the poorer fed sheep in Experiment 1 and only about one-sixth of that lost by the other two groups of infected animals. Indeed, judging by the changes in total circulating iron, it can be calculated that even if fully engaged into the system, storage iron in itself could not have serviced the plasma iron pool beyond/

beyond the 12th week in any of the animals concerned. Consequently, the relatively poor erythropoietic performance both of the animals restricted to hay and of the sheep in the second experiment when transferred to the lower plane of nutrition, must reside primarily at the level of iron absorption.

It can be appreciated from Figures 4 and 8 that 10 to 20-fold increases both in the amount and and efficiency of dietary iron utilisation were required of all infected animals to remain in positive iron balance. Although absorption was not measured directly, there can be little doubt that in the first experiment this was potentiated to a greater extent in the sheep given concentrates. Despite losses of almost 35 mg/day and a 4-fold increase in iron flow to the marrow, the plasma iron pool of this group was reasonably well maintained; by contrast, even the moderate increase in marrow iron supply exhibited by the group restricted to hay was achieved to the severe detriment of the plasma iron pool which began to deteriorate around the 10th week when iron losses were only 6 mg/day. It could be argued that such differences were simply a reflection of the better appetite and hence iron intake of the former. Whilst this was undoubtedly an important factor, the fact that the intake of both groups was comparable during the 5 weeks before necropsy suggests that the better erythropoietic response of the sheep given concentrates was also achieved through a greater efficiency of dietary iron utilisation.

This might be explained in a number of ways, e.g. by the presence of more "available" iron in the compound rations; by the absorption-enhancing action of proteins and amino acids in the intestinal/

intestinal lumen [59 - 61]; and finally, by the physiological condition of the animals themselves. Whilst the first possibility seems unlikely (apart from the small amount of ferrous sulphate in the mineral additive, the type of iron in all feedstuffs was probably fairly similar), it is now becoming increasingly recognised that proteins play a major part in promoting iron absorption in man and this effect appears to be mediated in two ways i.e. (a) via their role as complexing and reducing agents within the gut lumen, thereby minimising the formation of sparingly soluble, poorly absorbed forms of iron and (b) by their presence within intestinal mucosal cells, thereby facilitating iron transport to the blood stream [62]. Inasmuch as the ratio of protein:iron consumed by the sheep receiving concentrates was consistently 2 - 3 times greater than in those restricted to hay, it is possible that the more efficient iron utilisation, and hence ultimately the better erythropoietic response shown by the former, was attributable to reduced competition for iron between protein and other complexing agents. Likewise the relatively poor erythropoietic response of the sheep in the second experiment during the period of low protein feeding was associated both with a reduced iron intake and a 50% drop in the protein:iron ratio. However at present there are more theoretical arguments than experimental data to support the involvement of this mechanism and more definitive experiments on the interaction between dietary protein and iron absorption are clearly required.

SUMMARY

Radioisotopic methods for the measurement of blood volumes and red cell turnover were used to investigate (1) the earlier and more severe anaemia recorded in sheep infected with F. hepatica and fed only hay compared to similarly infected sheep receiving additional concentrates, and (2) the more rapid development of the anaemia in chronically infected sheep following their change from high to low protein feeding.

In sheep harbouring an average of 500 flukes the anaemia was caused by a combination of haemodilution, intra-hepatic and biliary haemorrhage. The earlier and more severe anaemia in protein-restricted sheep reflected the earlier development of these changes possibly associated with a faster rate of fluke migration through the liver. The ultimate severity of the anaemia was, however, dependent on the ability of infected sheep to replace red cells lost via biliary haemorrhage and this was found to be impaired in the poorer fed sheep as a result of iron deficiency.

Haemodilution did not contribute to the anaemia which developed in sheep harbouring 200 flukes and provided with a high protein diet these animals were able to maintain only slightly reduced PCV's and circularing red cell volumes despite substantial enteric red cell losses. Since, the severity of the biliary haemorrhage experienced by these sheep was unaffected by a change to low protein feeding, the subsequent decline in PCV's and circulating red cell volumes must have reflected a relative impairment to the erythropoietic response.

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CHAPTER 3

The Relationship of Host Nutrition and Fascioliasis: The aetiology of the hypoalbuminaemia which develops in sheep following infection with F. hepatica

INTRODUCTION

In addition to anaemia, sheep infected with F. hepatica invariably exhibit substantial changes both in the amount and distribution of their plasma proteins [1-3]. These changes, which are well exemplified by the results presented earlier, generally take the form of a depression in albumin relative to the globulins and develop virtually in two stages. The first of these coincides roughly with the period of fluke migration and is characterised by a progressive but usually mild hypoalbuminaemia, a much more pronounced hyperglobulinaemia, and hyperproteinaemia of variable severity. The second stage, which is associated with the presence of adult parasites in the bile ducts is attended by further deterioration in albumin levels as well as by progressive reductions in globulin and total protein concentrations. Although there is little disagreement on the nature of these changes, their aetiology has not escaped controversy. Since albumin accounts for up to 60% of the total plasma proteins and 80% of the colloid osmotic pressure, and is also ultimately involved in the transport of many important minerals and hormones [4], it is perhaps natural that most attention has been given to the question of how the liver fluke causes hypoalbuminaemia.

Most early workers attributed the condition to reduced albumin synthesis [1,5]. This was based on the assumption that because the liver of infected animals is so often severly damaged structurally [5], its function, which includes synthesis of albumin and other plasma proteins/

proteins [6] must also be seriously impaired. However, whilst infected animals do undoubtedly experience some degree of liver dysfunction, - this is reflected for example in high serum levels of certain enzymes known to be involved in protein metabolism within liver cells [3,7,8], and also in slow rates of bromsulphthalein clearance from the blood [9], it has yet to be demonstrated that these or any other disturbances adversely affect plasma protein production.

Indeed, there are a number of good arguments against this possibility, not least of which is that the above disturbances are generally restricted to the migratory stage of the infection and apparently disappear when the flukes enter the bile ducts. Significantly, it is during this latter stage of the disease that plasma protein concentrations deteriorate most and clinical evidence of the sequelae of hypoproteinaemia e.g. ascites, submandibular oedema and reduced blood mineral levels is most obvious [1,10,11]. This suggests that although albumin synthesis may well be reduced during the period of fluke migration, such a mechanism could not in itself account for the more pronounced hypoalbuminaemia observed subsequently.

Since protein concentrations are essentially determined by the relative rates of addition to and withdrawal from the circulation by synthesis and catabolism respectively, the only proper way to approach the problem is to compare these rates in infected and worm-free animals i.e. examine the "turnover" of the protein under investigation. Unfortunately there is no suitable direct method for measuring plasma protein synthesis in ruminants, but catabolism is easily determined using proteins labelled <u>in vitro</u> with one of the radioactive isotopes

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of iodine [6]; provided care is taken with their preparation such molecules behave in exactly the same way as unlabelled molecules [12].

Plasma proteins labelled with radioiodine were first applied to the study of fascioliasis by Dargie and Nansen and their colleagues [13,14], who showed that animals infected with adult parasites were in a hypercatabolic state with respect to both albumin and immunoglobulin, and that in all cases this commenced around the time that the flukes entered the bile ducts. It was also associated with a high excretion of isotope in the faeces, indicative of excessive plasma loss into the gut. Such losses could not be quantified using radioiodinated proteins due to reabsorption of radioiodide from degraded molecules, but subsequent work with proteins labelled with isotopes not normally absorbed from the gut i.e. ^{95}Nb and ^{51}Cr, as well as with labelled macromolecules resistant to enzymic degradation (e.g. ^{131}I-polyvinylpyrrolidone), confirmed their overriding importance in this disease [13,15-18]. At the same time these studies also demonstrated that the loss of plasma was substantially greater than could be accounted for by the passage of whole blood into the gut, implying that in addition to the losses sustained through the parasites' haematophagic activities, there was considerable plasma leakage through the damaged biliary epithelium; this was later confirmed by the presence of electron dense material between epithelial cells lining the biliary mucosa [19].

As mentioned earlier, iodine-labelled proteins are particularly suitable for measuring rates of catabolism, but they also have two further advantages in metabolic studies. Firstly, if the plasma concentration/

concentration of the protein concerned is known, they can be used to estimate the amount of that protein within the different body compartments and hence its distribution between these compartments [20,21]. Bearing in mind that large quantities of the plasma proteins are present extravascularly, this is a particularly useful characteristic (a) because situations can exist where the concention of a given protein in the blood is reduced, but because of an increase in plasma volume, the total amount is normal e.g. in ovine schistosomiasis [22], and bovine trypanosomiasis [23]; and (b) because in many parasitic infections, including fascioliasis, reductions in the circulating pool are often smaller than occur extravascularly and therefore the ratio of intra- to extravascular protein is lower [24]. Needless to say, neither of these situations can be detected simply by measuring protein concentration.

The other main advantage of radioiodinated proteins is that synthesis can be inferred indirectly from changes in rates of catabolism and pool sizes [22]. Although absolute significance cannot be attached to the figures obtained, the procedure does nevertheless provide an insight into a process which cannot be examined otherwise and therefore has some application. When assessed in this way, most authors have found that animals suffering from chronic fascioliasis are able to counteract the accelerated breakdown or loss of plasma proteins through increased synthesis. This is most noticeable in the case of the immunoglobulins, but is also true of albumin [13,14].

From the above account it is clear that underlying all the changes in plasma protein concentration associated with fascioliasis are/

are disturbances in the balance between catabolism, distribution and synthesis. Unfortunately the extent to which any or all of these factors is involved in a given individual has never been examined throughout the disease process nor compared in animals harbouring different fluke populations or maintained on different planes of nutrition. Consequently our understanding of the disease itself, of how this is modified by these variables, and of how the host adapts to changes in protein intake arising from inappetence and/or variations in dietary quality is either incomplete or non-existant.

In the first chapter of this thesis, it was shown that fluke infected sheep fed hay alone experienced more rapid reductions in serum albumin concentrations than animals with similar fluke burdens but supplemented with a high protein compound ration. It was also shown that sheep removed from a high to low protein diet suffered a more rapid deterioration in albumin levels following the change. The work reported in this chapter was designed to investigate the changes in albumin metabolism underlying these observations.

MATERIALS AND METHODS

Experimental Animals and Design

Detailed descriptions of the sheep and diets used in the two experiments and of the experimental designs are given in chapter 1. In the first experiment 16 sheep were divided into two equal groups and fed either hay alone or hay plus a high protein compound ration. Five sheep in each group were individually infected 6 weeks after the start of the investigation with 1,000 F. hepatica metacercariae, while the remainder acted as pair-fed controls to 3 of the infected animals. Radioiodinated albumin was given intravenously at intervals during the study to measure albumin pool sizes and catabolic rate, and ^{51}chromic chloride to quantify plasma and albumin losses into the gastrointestinal tract.

In the second experiment similar measurements were made on 12 sheep, half of which were each infected with 600 F. hepatica metacercariae, the remainder acting as pair-fed controls. During the first 16 weeks of infection the diet consisted of a high protein compound ration, but this was replaced by a low protein ration for the remaining 4 weeks of the study.

All animals were confined in standard metabolism cages and allowed free access to water. Each day the total outputs of urine and faeces were collected and to ensure the rapid excetion of radioactive iodine from degraded protein, 10ml 0.75% KI was given daily as a drench. Body weights were recorded twice weekly and serum samples taken for biochemical analysis at similar intervals.

Biochemical Methods

Total serum protein and albumin concentrations were determined by the methods described earlier.

Labelling, Injection and Sampling Procedures

Solutions of ^{125}I-labelled albumin and ^{51}chromic chloride prepared as described previously and containing respectively about 500μCi and 1mCi, were administered via a jugular catheter to each animal. Blood samples were collected 15 minutes after injection, twice daily over the following 3 days and thereafter daily until re-injection or necropsy. Aliquots of the plasma and the daily urine and faecal collections were prepared for the measurement of radioactivity as described earlier. Standard solutions of each radioisotope were prepared by diluting aliquots of the solutions used for injection.

Radioactivity Measurements

Radioactivity determinations on plasma, urine and faeces were carried out as described previously. The activities due to ^{51}Cr and ^{125}I in samples containing both isotopes were determined by γ-ray spectrometry and the application of "overlap" factors calculated from the count rates of their respective standard solutions. These solutions were also used to correct for radioactive decay.

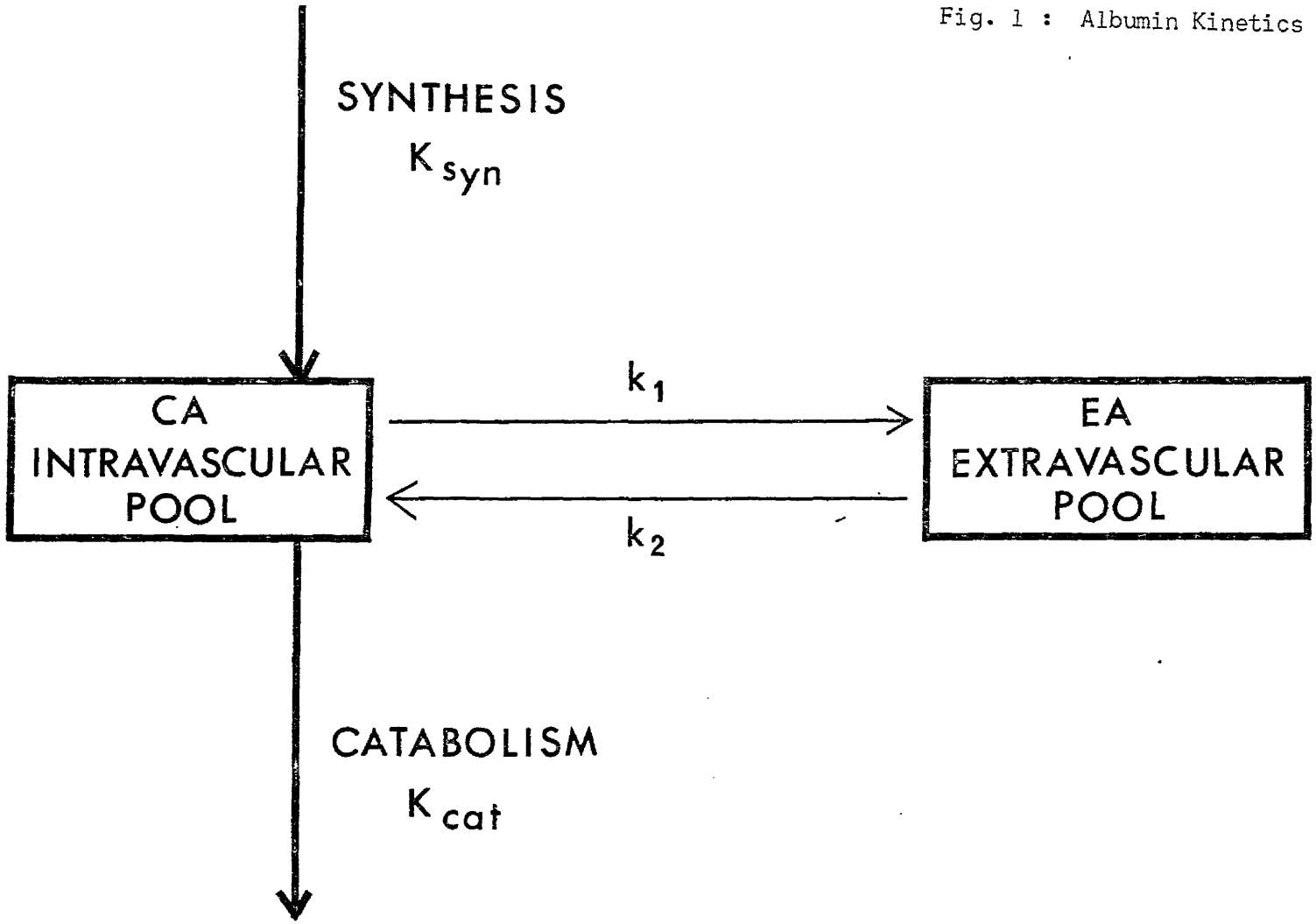
Measurements of Albumin Distribution and Catabolism

Albumin is continually entering and leaving the vascular system/

system and therefore to understand the cause of any change in serum albumin concentration it is necessary to study the metabolism of this protein. The measurements of albumin kinetics made in these experiments were based on the simplified "two compartment" model illustrated in Fig. 1. This model assumes that albumin passes from the intravascular pool (CA) through pores in the capillary walls into one common extravascular pool (EA) and returns via the lymphatics. This assumption is an oversimplification since there are at least two main groups of extravascular pools each of which equilibrates with the plasma at a different rate [25]; K_1 and K_2 therefore reflect the average rates of movement between the pools. The model also assumes that synthesis (K_{syn}) and catabolism (K_{cat}) of albumin take place intravascularly. This assumption appears to be valid since albumin is synthesised exclusively in the liver and upon release from the parenchymal cells enters the hepatic lymph or venous blood [6]. The sites of albumin catabolism, on the other hand, are not known although the liver, kidney and gastrointestinal tract have been proposed [4], but only "intravascular" breakdown fulfils the requirement of the constant day to day degradation established for this protein [21]. Theoretically for absolute significance to be attached to determinations based on such a model it is necessary for the animal to be in a steady state, i.e. for the pools to remain unaltered and for K_{syn} and K_{cat} to be equal during the measurements. While this situation clearly does not exist in parasitised animals important information can nevertheless be obtained particularly when the studies are of a comparative nature.

Albumin/

Fig. 1 : Albumin Kinetics



Albumin trace-labelled with radioiodine, by the method described earlier, has a number of characteristics which make it especially suitable for metabolic studies. Firstly, it behaves metabolically like the animals own unlabelled molecules; secondly, the label remains firmly attached to protein until it is degraded; and finally, when the protein is catabolised the isotope is not reutilised in protein synthesis but quantitatively and rapidly excreted in the urine provided the thyroid has been blocked by prior administration of excess stable iodide [12]. Hence, by determining the radioactivity in plasma, urine and faeces following the intravenous injection of labelled albumin, pool sizes and catabolic rate can be measured.

Intravascular Albumin Pool (CA)

This was estimated from the plasma volume (Vp), determined by application of the dilution principle (see chapter 2), and the serum albumin concentration at the time of each injection of labelled albumin:

i.e. CA (g) = Vp (ml) x serum albumin (g/ml)

Total Body Albumin Pool (TA)

Total body albumin was determined by the "extrapolation" method of Sterling [20], and by the "equilibrium time" method of Campbell and his colleagues [21].

(i) Extrapolation Method

^{125}I plasma activities (Q_p) expressed as a percentage of the 15 minute sample were plotted on a semi-logarithmic scale against time after injection (Fig. 2). The plasma activity fell rapidly at first/

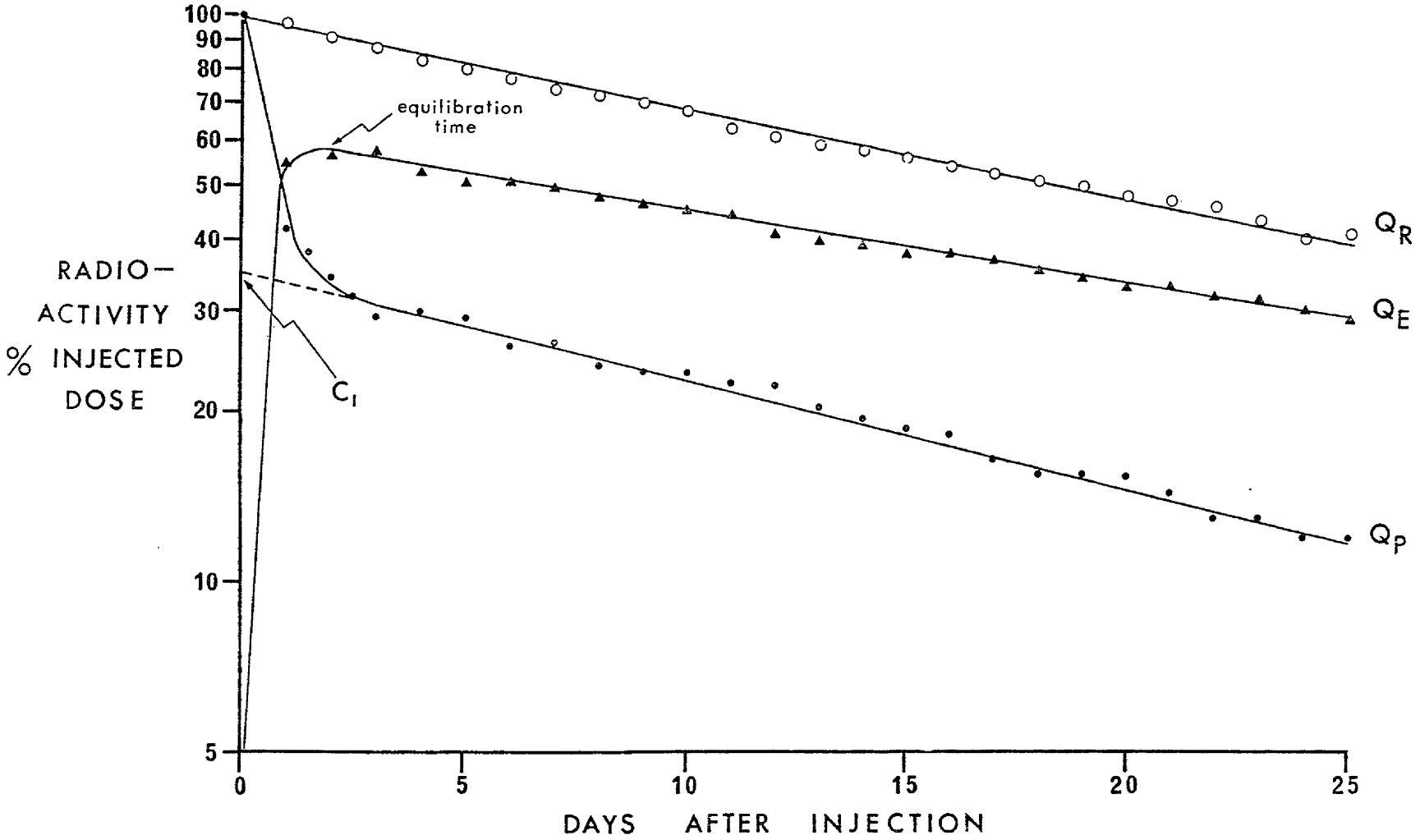


Fig. 2 : Turnover of ^{125}I-labelled Albumin in a Sheep.

first, due primarily to the movement of the labelled protein from the circulation into the extravascular compartment, but then declined exponentially. Sterling considered the slope of this latter portion of the plasma curve to be due solely to catabolism and therefore that its extrapolation back to the ordinate (intercept C_1) gave the fraction of the total albumin present in the plasma if mixing of the label between all the body pools had taken place immediately:

$$\text{i.e. } TA\ (g) = \frac{CA\ (g)}{C_1} \times 100$$

However, the distribution of albumin between the intra- and extravascular compartments is a continuous process and since the activity in the plasma is being reduced by catabolism of labelled albumin and addition of freshly synthesised unlabelled protein, there is a net flow of ^{125}I-albumin from EA (which is unaffected by these processes) to CA. Consequently the slope of Q_P is reduced, C_1 is underestimated and TA overestimated.

(ii) Equilibrium Method

The total body radioactivity (Q_R) at the end of each day, was obtained by subtracting the cumulative activity excreted in the urine and faeces from the total injected activity. Expressed as a percentage of the injected activity this value was plotted semi-logarithmically against time and the extravascular activity (Q_E) obtained indirectly as the difference between Q_R and Q_P at the end of each day (Fig. 2). The extravascular activity increased to a maximum value at which point it was assumed that an equal amount of ^{125}I-albumin/

^{125}I-albumin was entering and leaving the extravascular pool, i.e. the system was in equilibrium, and hence the ratio Q_E/Q_P = EA/CA or

$$TA = \frac{CA\ (Q_P + Q_E)}{Q_P}$$

This method is theoretically more acceptable since it takes into account intravascular catabolism and redistribution of labelled material. It does however have two major drawbacks in that (a) it is technically more demanding, requiring the quantitative collection of urine and faeces and (b) selection of the point of maximal extravascular activity is rather difficuilt.

Both methods were utilised in these studies and since the values obtained were not significantly different, the mean figure from both procedures is presented in the text.

Extravascular Albumin Pool (EA)

The extravascular albumin pool was determined as the difference between the total body and intravascular pools

i.e. EA = TA - CA

Absolute values expressed on a body weight basis were used to compare albumin pool sizes.

Albumin Catabolism and Synthesis

The slope of the linear part of the plasma activity curve, Q_p, (Fig. 2) although not solely due to catabolism is nevertheless a rough approximation and therefore provides a useful semi-quantitative index of catabolism. The rate of degradation based on this line is usually called the "apparent albumin half-life" and is calculated as the time taken for the plasma activity to fall by 50%.

A/

A more accurate measure of catabolism was devised by Campbell and his colleagues [21], based on an analysis of excreted radioactivity. This method assumes that catabolism takes place in the intravascular compartment and that liberated isotope is rapidly and quantitatively excreted. Under such circumstances the excreted activity represents a certain fraction, F(CA), of the labelled albumin present within the circulation. This fraction was calculated from the plasma activity and the total activity excreted during the subsequent 24 hours.

$$F(CA) = \frac{\text{Total urine activity + Total faecal activity}}{\text{Plasma activity/ml x Vp}}$$

Conversion of fractional catabolic rates to absolute amounts of albumin degraded was then made using the equation:

$$\text{Albumin catabolised (g/day)} = CA(g) \times F(CA)$$

Albumin synthesis over any given period was then calculated from the sum of albumin degraded and the change in the total exchangeable pool.

Estimation of Plasma and Albumin Leakage into the Gut

Excessive losses of plasma and hence of albumin into the gastrointestinal tract via the bile are a recognised feature of fascioliasis [13,16]. Some indication of the extent of such losses may be obtained with ^{125}I-albumin by dividing the total activity excreted in the faeces over 24 hours by the activity/ml plasma at the beginning of the collection period. The figure obtained, which is known as a "clearance", represents the volume of plasma which theoretically must have passed into the gut during that period to give rise to the faecal radioactivity./

radioactivity. However, since substantial amounts of the isotope from labelled protein degraded within the gut are reabsorbed and subsequently excreted in the urine [26], such values grossly underestimate protein losses by this route and therefore can only be used as a qualitative guide.

For a more accurate estimate of enteric plasma leak, it is necessary to use a marker which is quantitatively excreted in the faeces on oral administration. Since it has been adequately demonstrated that no significant reabsorption of ^{51}Cr occurs from the alimentary tract of most species [27-29], this isotope fulfils this particular criterion. Albumin labelled *in vitro* with ^{51}Cr as $^{51}CrCl_3$ was first introduced by Waldmann [27], to measure protein loss into the gut, but later studies [30,31] revealed that following injection of such preparations much of the isotope was rapidly lost from the protein and was either excreted or became bound to other proteins in the plasma. However, this feature is not a serious drawback provided it can be assumed that the losses into the gut are of whole plasma.

Since it has been shown that the rate of disappearance of ^{51}Cr from the plasma is the same irrespective of whether it is administered as $^{51}CrCl_3$, ^{51}Cr-albumin or ^{51}Cr-serum [31], the studies described in this chapter were conducted using $^{51}CrCl_3$ injected directly into the circulation [32]. Daily faecal "clearances" of plasma were calculated as described for ^{125}I, which, together with the appropriate serum albumin concentration, enabled estimation of enteric albumin losses:

i.e. $$\text{Albumin lost into the gut (g/day)} = \text{Serum albumin (g/ml)} \times {}^{51}\text{Cr-plasma "clearance" (ml/day)}$$

RESULTS

EXPERIMENT 1

Two groups of Scottish Blackface wethers were maintained on diets consisting of chopped hay or chopped hay and a high protein compound diet. Six weeks later, 5 animals in each group were individually infected with 1,000 F. hepatica metacercariae and together with 3 worm-free "pair-fed" controls measurements of the distribution and metabolism of albumin, and the intestinal plasma loss made during the following 15-20 weeks. The results presented below are the mean values recorded for the pair-fed sheep; individual data are given in Appendix 3.

The salient features of the serum albumin changes and protein intakes of the animals concerned were described in detail in chapter 1, but the mean results are combined in Fig. 3 because of their relevance to the studies reported below.

Albumin Pools

The results of the measurements of albumin pools are illustrated in Fig. 4a and b. When placed on their respective diets the amount of protein in each compartment was similar in all animals, but during the following 6 weeks, sheep on the better diet increased their intra- and extravascular pools in parallel with growth while those restricted to hay experienced slight reductions; as a result the total body pool of albumin in the former was about 0.3g/kg (10%) greater at the time of infection ($p < 0.001$). Infected sheep on each diet suffered reductions/

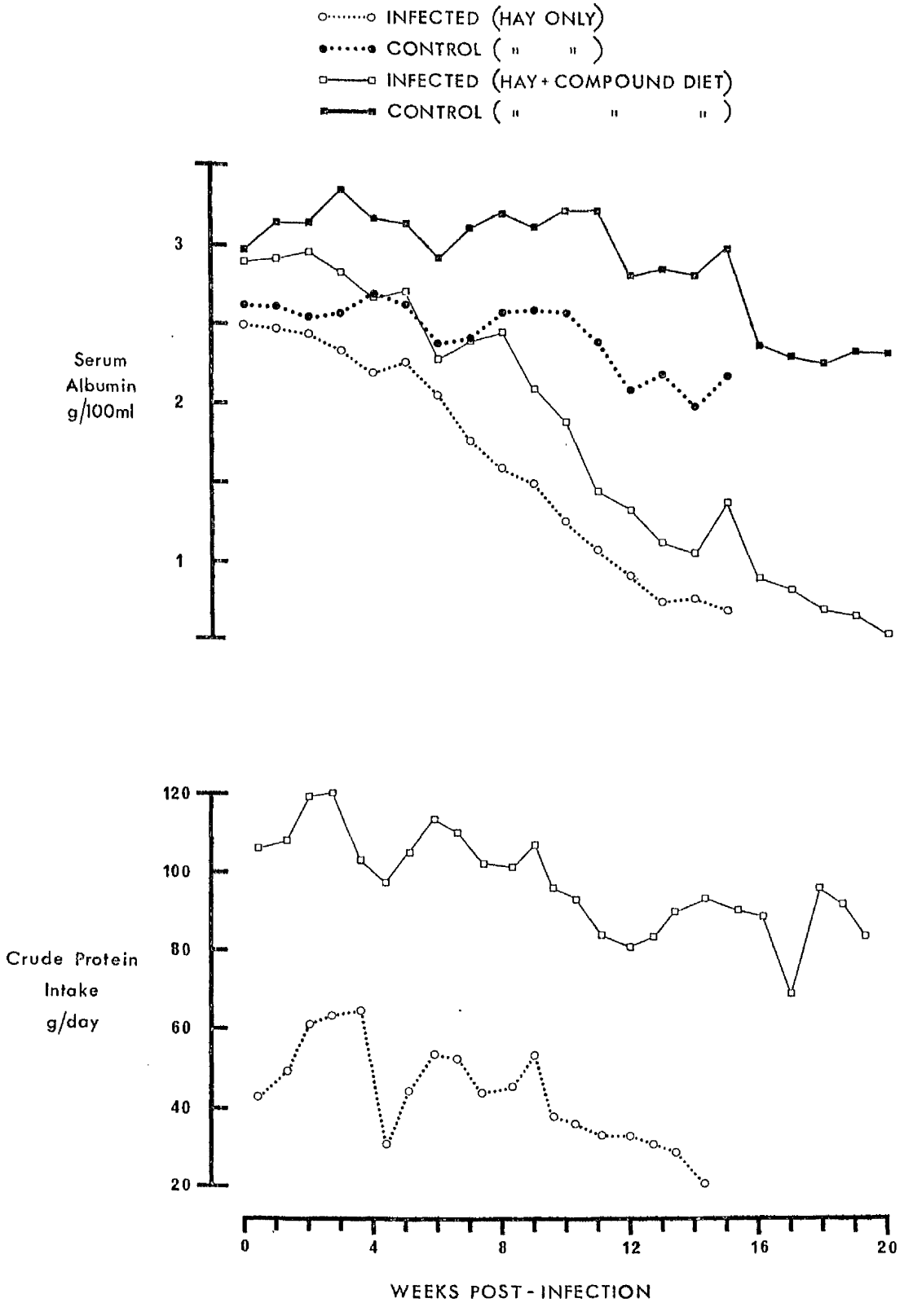


Fig. 3 : Serum Albumin Levels and Crude Protein Intakes of Fluke-Infected and Control Sheep.

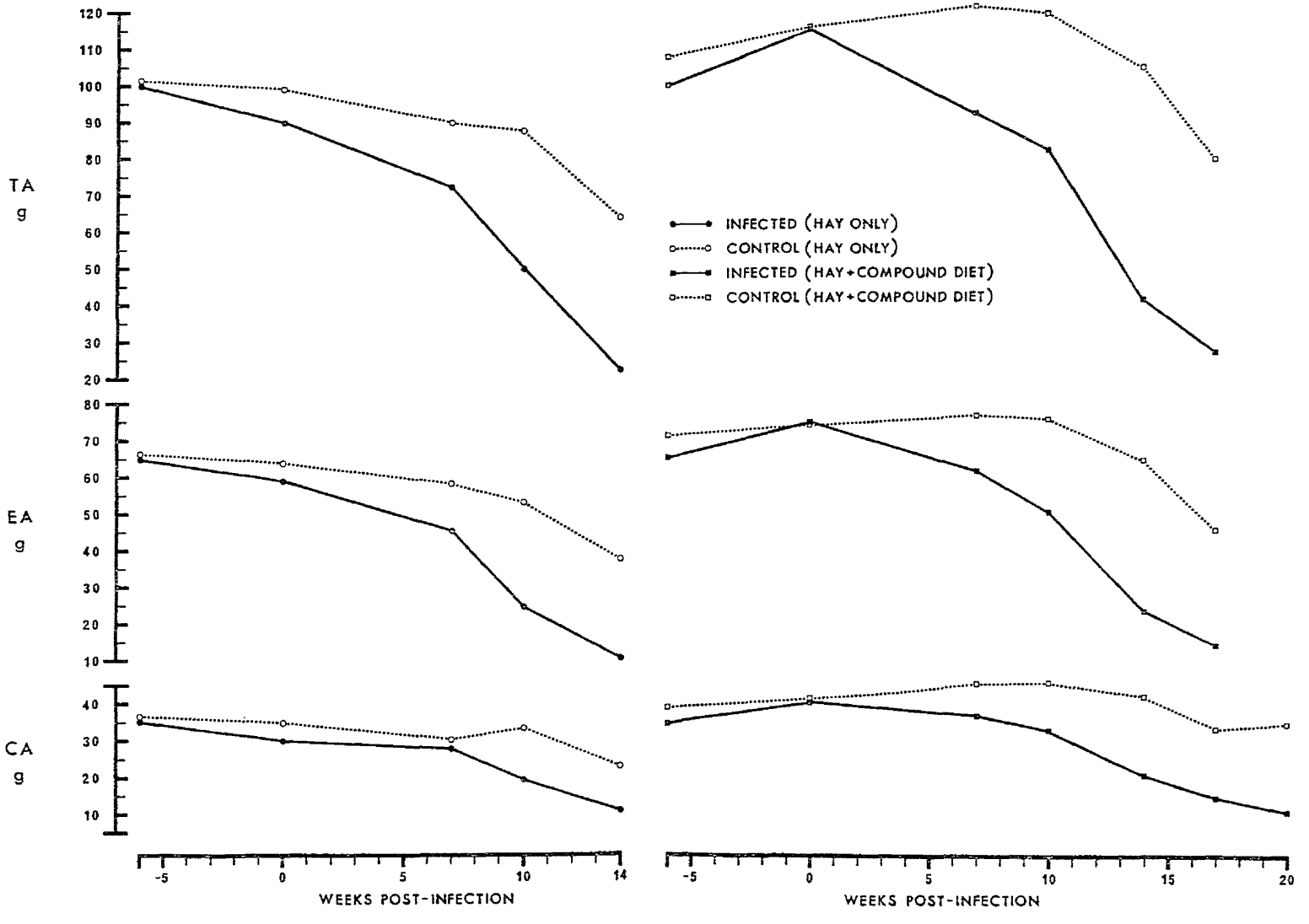


Fig. 4a : Effect of Diet on the Albumin Pools of Fluke-Infected and Control Sheep.

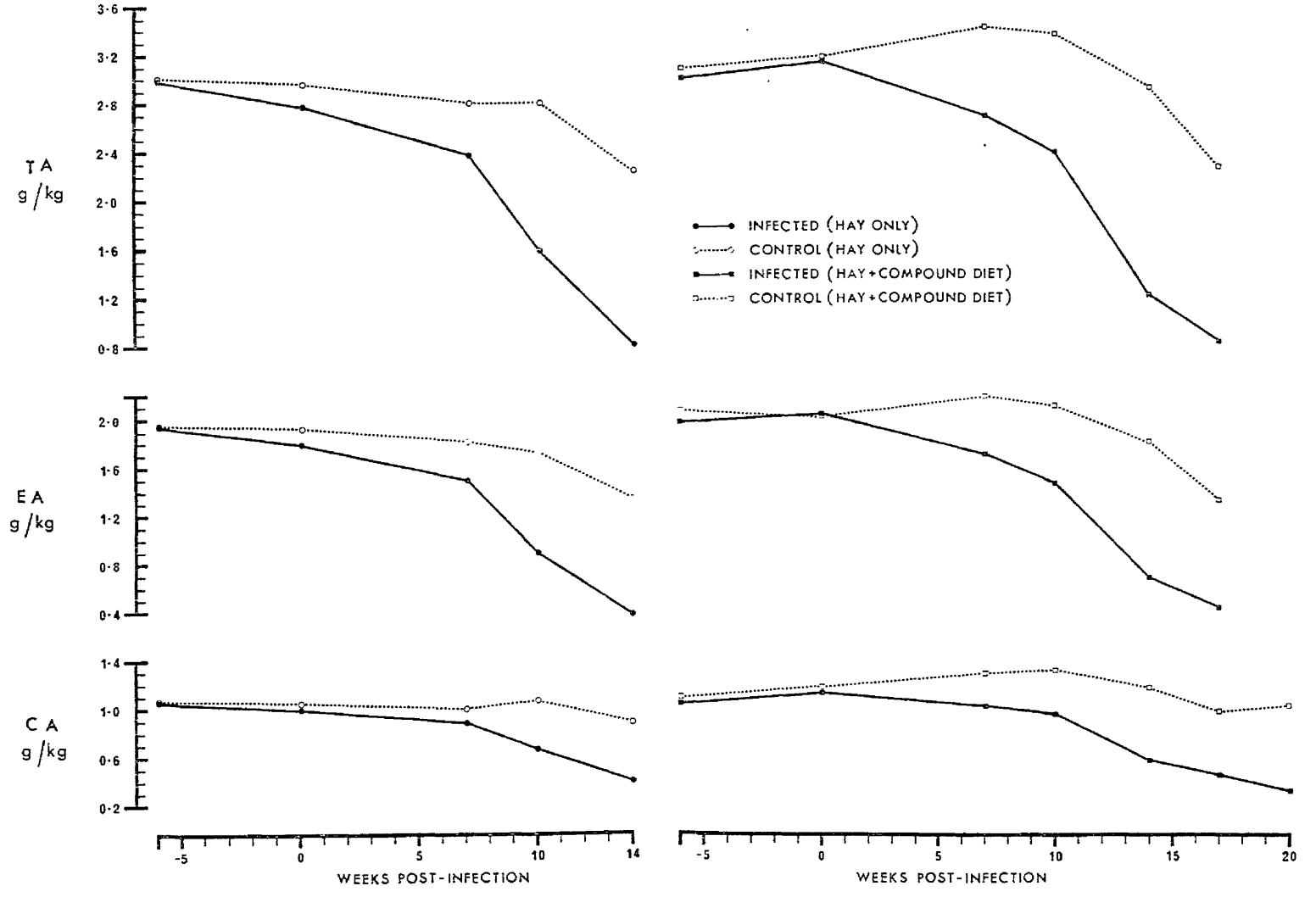


Fig. 4b : Effect of Diet on the Albumin Pools (related to body weight) of Fluke-Infected and Control Sheep.

reductions in the size of both pools during the following 7 weeks but these were slightly more pronounced in the group restricted to hay whose total body pool fell by 20% as compared with 14% in the supplemented sheep. By the 10th week the difference between these animals was even more marked, the total amount of albumin in the former having been reduced by almost 50% (1.34g/kg) and that of the latter by only 25% (0.76g/kg) relative to pre-infection values. A further point of interest is that because of concurrent increases in plasma volume (these were particularly dramatic in the hay only group during the first 7 weeks, and in the high protein group between the 7th and 10th weeks), reductions in the intravascular pool of both groups up until this time (15%) were small in comparison with those recorded for serum albumin concentration (55% and 35% respectively).

By the 14th week, the amount of albumin in each group was about one half of that recorded on the 10th week, the high protein group having suffered substantially greater losses during this period, and when the measurements were repeated on the 17th week, pool sizes of the supplemented sheep were almost identical to those recorded for the poorer fed animals 3 weeks earlier, each group having suffered total reductions in the order of 75%, of which almost two thirds was lost from extravascular sites. This latter point is clearly illustrated by the fall in EA/CA ratios as the infections progressed (from 1.8 to 0.9). Extravascular albumin was not measured in the survivors at the 20th week, but since the intravascular pool was about 35% lower than at 17 weeks, it is almost certain that this was also further depleted./

depleted. The only change of any note in the control sheep during the first 10 weeks was a minimal (5%) drop in the total body albumin of the poorer fed animals, but by the 14th week this had deteriorated by 30% (about 0.7g/kg) relative to that recorded at the outset. Although the supplemented animals exhibited lesser reductions during this period (about 12% or 0.4g/kg), these were more marked subsequently with the result that by the 17th week the total albumin pool of this group was depleted to an extent (35%) similar to that found earlier in their poorer fed counterparts. As in the infected animals, both pools contributed to the deterioration in albumin status, but by far the major loss occurred extravascularly.

Albumin Catabolism and Synthesis

The data shown in Fig. 5 are the results of the measurements of albumin degradation. Although noticeably higher in the poorer fed sheep (25 days as compared with 20 days), albumin half-lives ($t_{\frac{1}{2}}$'s) of all animals remained steady before and during the first 7 weeks of infection, thereafter declining in the infected sheep and increasing in the controls. Both changes were initially more pronounced in the animals restricted to hay, $t_{\frac{1}{2}}$'s falling to 12 days in the infected and rising to 40 days in the control groups between the 7th and 14th weeks, but equally dramatic alterations occurred subsequently in the supplemented sheep, with values dropping to 6 days and lengthening to 35 days by week 20.

Equally/

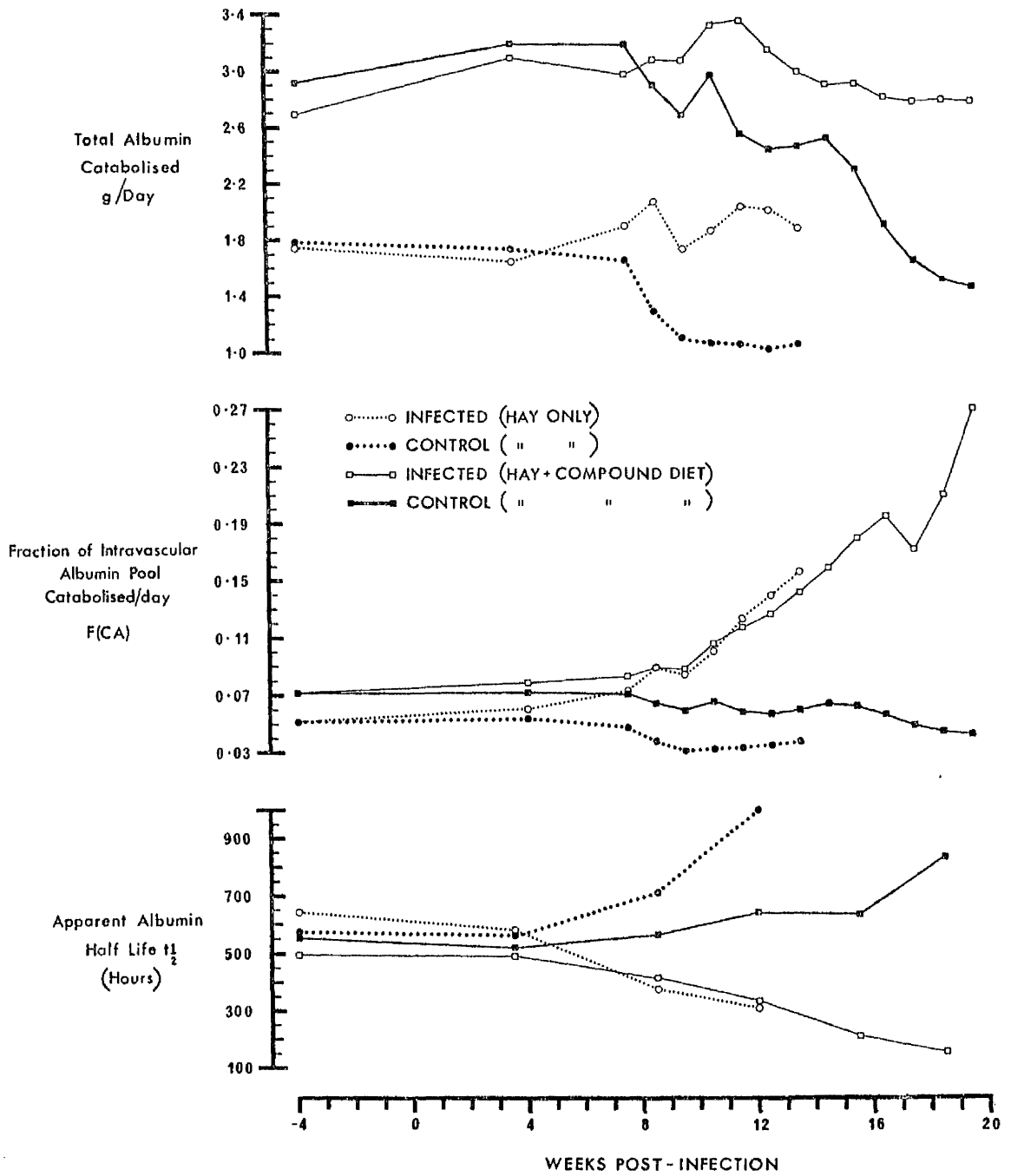


Fig. 5 : Effect of Diet on the Catabolism of Albumin in Fluke-Infected and Control Sheep.

Equally spectacular were the accompanying changes in the fraction of the intravascular pool (F(CA)), and the absolute amounts of albumin degraded each day. Fractional catabolic rates, which had fluctuated around 5% and 7% respectively in the low and high protein groups until the 7th week of infection ($p < 0.01$), increased dramatically thereafter, reaching about 15% in all infected animals by the 14th week and 25% in the better fed sheep by week 20. By contrast, the corresponding control figures declined gradually between the 7th and 14th weeks in those restricted to hay; a similar hypocatabolism (40%) developed in the protein-supplemented sheep during the final 6 weeks of the experiment. Significantly, despite their much higher fractional rates of catabolism, the mass of albumin degraded by infected sheep increased by only 20%, and in the case of the controls it actually fell to about one half of that recorded beforehand; also noteworthy was that the amounts catabolised by sheep given concentrates were significantly higher throughout the investigation ($p < 0.001$).

The results of the estimations of albumin synthesis made at various stages of the investigation are given in Table 1. Firstly, the obvious correlation between rates of albumin synthesis and protein intake in all animals before infection and in the controls throughout the study; these rates were always significantly higher in the sheep supplemented with concentrates ($p < 0.01 - 0.001$). Secondly, the lower rates of synthesis in both infected groups, but more particularly/

Table 1: Albumin Synthesis (mg/kg/day ± S.E.) in Sheep Infected with *F. hepatica* and Pair-fed Controls

	Hay Only		Hay + Compound Diet	
	Control	Infected	Control	Infected
Pre-Infection	48.6 ± 6.2	46.2 ± 9.2	87.1 ± 7.9	89.0 ± 2.8
0 - 7 Weeks After Infection	48.1 ± 7.4	39.5 ± 5.9	91.9 ± 5.5	74.8 ± 6.3
7 - 14 Weeks After Infection	20.5 ± 1.7	30.5 ± 8.0	66.7 ± 6.2	55.7 ± 10.9
14 - 17 Weeks After Infection			28.1 ± 5.8	64.3 ± 14.1

particularly in the animals restricted to hay, during the first 7 weeks, relative both to that recorded beforehand (28% as compared with 15%), and to the corresponding values for the pair-fed controls (21% and 18% respectively). Finally, the relatively better synthetic capacity of the infected sheep during the biliary stage of the disease, a feature noted earlier in the poorer fed group.

Passage of Plasma and Albumin into the Gastrointestinal Tract

In order to examine further the aetiology of the above changes, and in particular the role of albumin loss into the gut, faecal "clearances" of plasma were calculated from the plasma and faecal ^{51}Cr radioactivities (Fig. 6). The control values remained steady throughout while those of the infected sheep increased progressively from about the 7th week and at least initially, at a slightly faster rate in the animals restricted to hay. These latter animals suffered a tenfold elevation in enteric plasma leak between the 7th and 14th weeks (from 25ml to 250ml/day), and although the losses experienced by their better fed counterparts were noticeably smaller during this period (35ml increasing to 190ml/day), they were ultimately much more severe (380ml/day by week 20). In other words the total amount of plasma lost by the poorer fed animals before the disease proved fatal was only about one half that experienced by those receiving concentrates (6 litres and 13 litres respectively) but in both cases substantially higher than expected from the haemorrhage recorded in the previous chapter (i.e. 3 litres and 8 litres respectively).

Also/

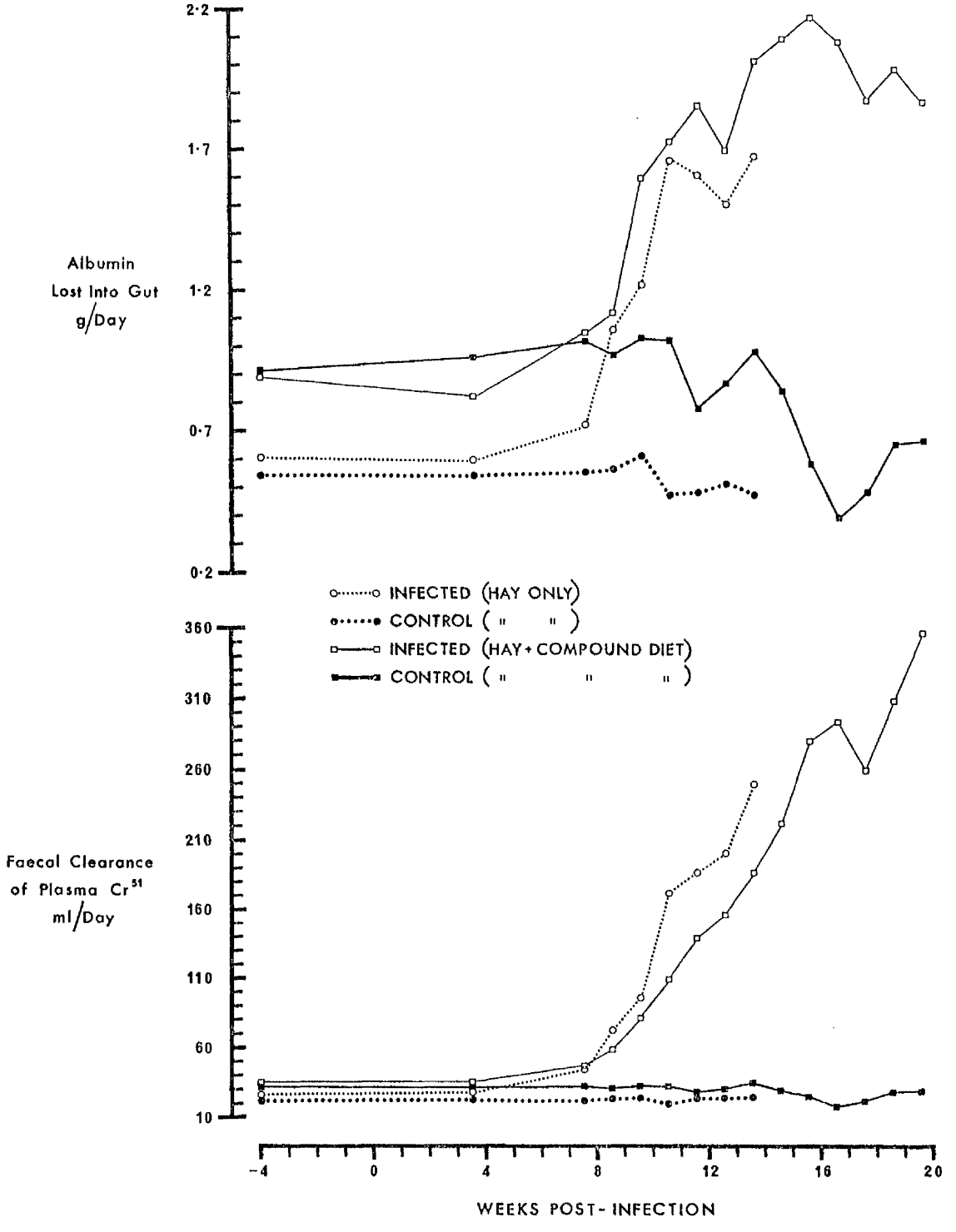


Fig. 6 : Effect of Diet on the Enteric Losses of Albumin and Plasma in Fluke-Infected and Control Sheep.

Also noteworthy was that in the control sheep significantly more plasma passed into the gut of the better fed group ($p < 0.001$).

These results clearly demonstrate that the hypercatabolism experienced by infected sheep was closely associated with excessive loss of plasma and hence albumin into the gut. The enteric albumin loss which can be estimated by relating the ^{51}Cr "clearances" to the appropriate serum albumin concentration are also depicted in Fig. 6. Before and during the first 6-8 weeks of infection the amount of this protein lost via the gut was similar in all animals within each nutritional group but higher in the sheep fed the higher protein ration (0.9g and 0.6g/day respectively, $p < 0.001$). These losses subsequently increased in the infected sheep but because of their more severe hypoalbuminaemia, the greater plasma leak recorded initially in the poorer fed group was not accompanied by a higher rate of albumin loss - in fact between the 7th and 14th weeks this increased to a similar level in both groups (about 1.7 g/day); likewise, despite a twofold increase in enteric plasma loss between the 14th and 20th weeks in the high protein group, the amount of albumin lost by this route increased by only 20% to 2g/day. Control figures on the other hand generally declined after the 8th week, particularly in the better fed animals which by the 17th week were losing albumin at a rate comparable to that of their poorer fed counterparts 3 weeks earlier (about 0.5g/day).

EXPERIMENT 2

Six Scottish Blackface wethers were each given 600 F. hepatica metacercariae, and together with 6 worm-free sheep acting as pair-fed controls, were maintained on a high protein ration (13 -15% crude protein) during the first 16 weeks of infection and on a low protein diet (8% crude protein) for a period of 4 weeks thereafter. Albumin pools were measured at intervals during the study and the catabolism of albumin and intestinal plasma loss estimated daily. For ease of presentation only the mean values for infected and control groups are depicted in the figures but individual data are presented in Appendix 3.

The serum albumin values recorded during this experiment and their relationship to protein intake are shown in Fig. 7; these have already been described in detail and therefore do not merit further comment at this stage.

Albumin Pools

The results of the measurements of total body albumin and its distribution are illustrated in Fig. 8. The changes recorded were of a similar nature to those described earlier, but much less severe. Before infection the amount of total exchangeable albumin was the same in both groups (but 16% and 35% higher, $p < 0.01$, than recorded for the high and low protein groups of the first experiment), and apart from a slight reduction (8%) in the infected animals, remained essentially unaltered during the first 8 weeks. However, by the 16th/

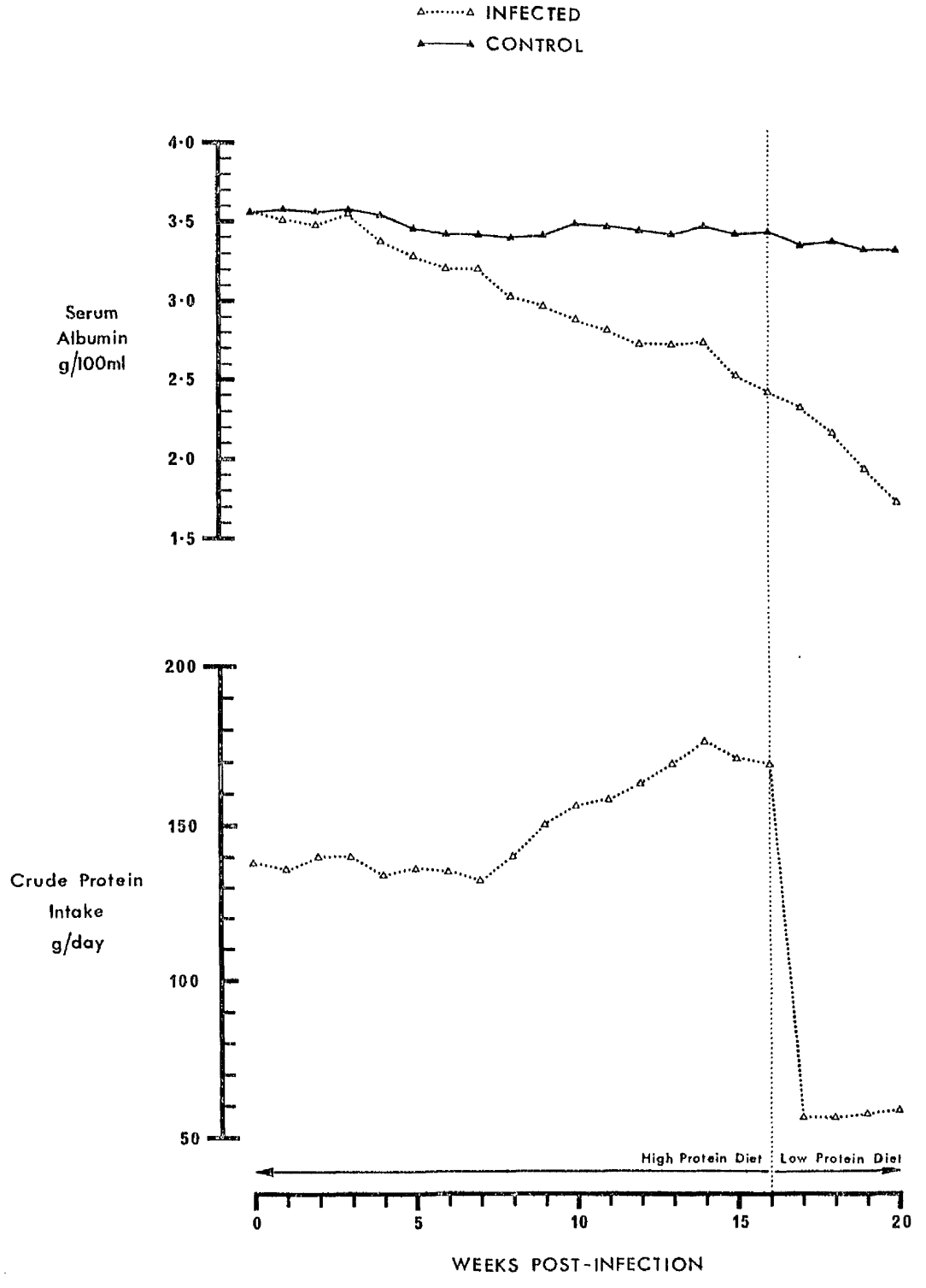


Fig. 7 : Serum Albumin Levels and Crude Protein Intakes of Fluke-Infected and Control Sheep.

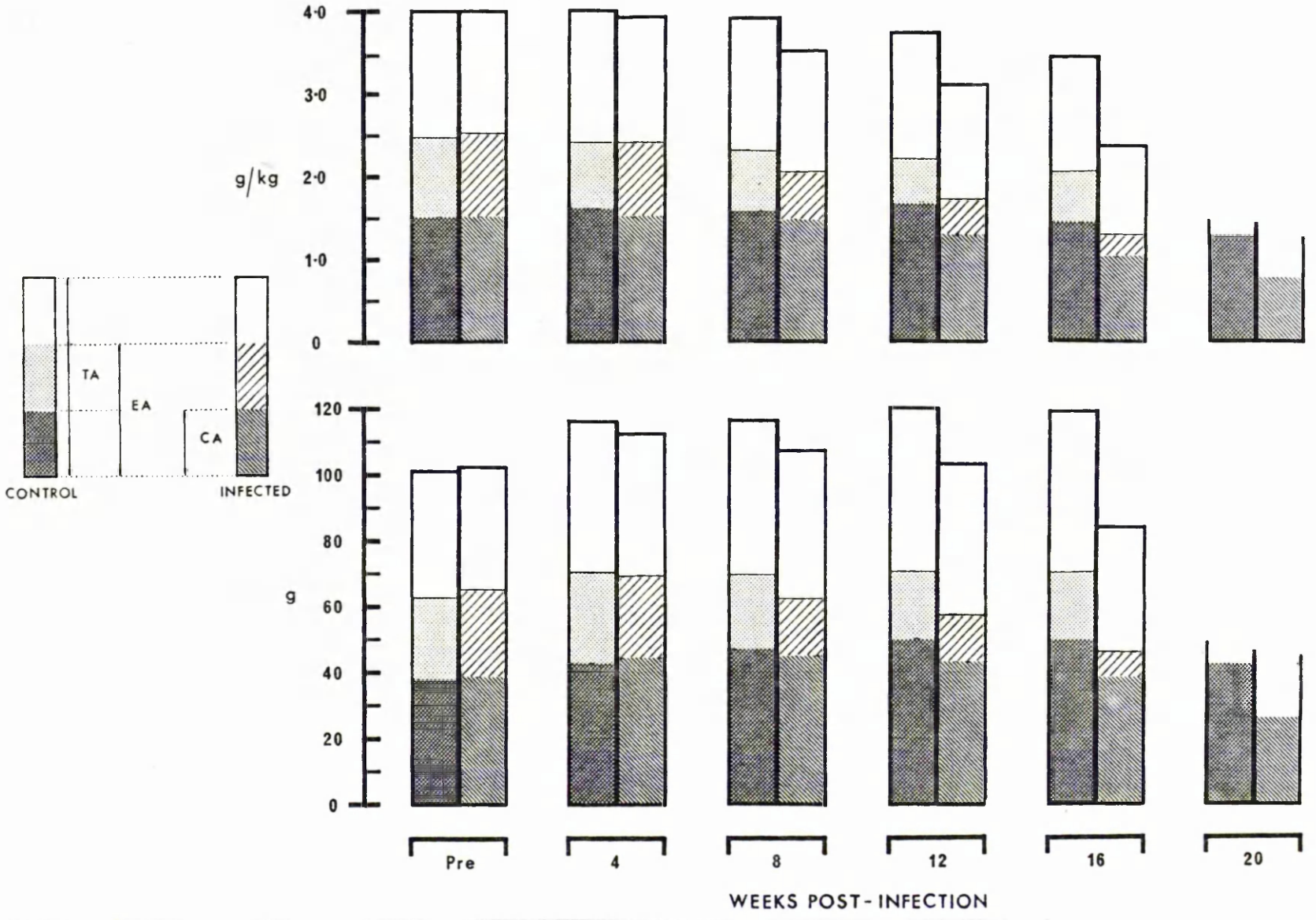


Fig. 8 : Effect of Diet on the Albumin Pools of Fluke-Infected and Control Sheep.

16th week the total amount of albumin in the infected group was 40% lower than at the outset and 30% lower than that of the controls ($p < 0.001$) which also experienced some depletion (10%) during this period; as in the previous experiment extravascular albumin was depleted to a greater extent EA/CA ratios falling from 1.7 to 1.2 in the infected sheep and from 1.6 to 1.4 in the controls. Unfortunately, the size of the extravascular pool was not determined following the change of diet, but judging by the figures obtained 4 weeks later for the intravascular pool (this fell by 30% and 13% respectively in the infected and control animals), it is certain that this was depleted in both groups but to a greater extent in the latter.

Albumin Catabolism and Synthesis

The results of the measurements of albumin catabolism, assessed in terms of the plasma half-life ($t\frac{1}{2}$) of ^{125}I-labelled albumin, and the fraction of the intravascular pool F(CA), and total amount of albumin degraded each day are summarised in Fig. 9. These show that between the 8th and 12th, and more particularly between the 12th and 16th weeks, the survival of labelled albumin in the circulation of infected sheep declined progressively (from 20 days prior to the 8th week to 12 days between the 12th and 16th weeks). This was accompanied by equally marked elevations in both the fraction of the intravascular pool and total amount of albumin degraded (from 8% to 14% and from 3.7g to 5.3g/day respectively). The corresponding values/

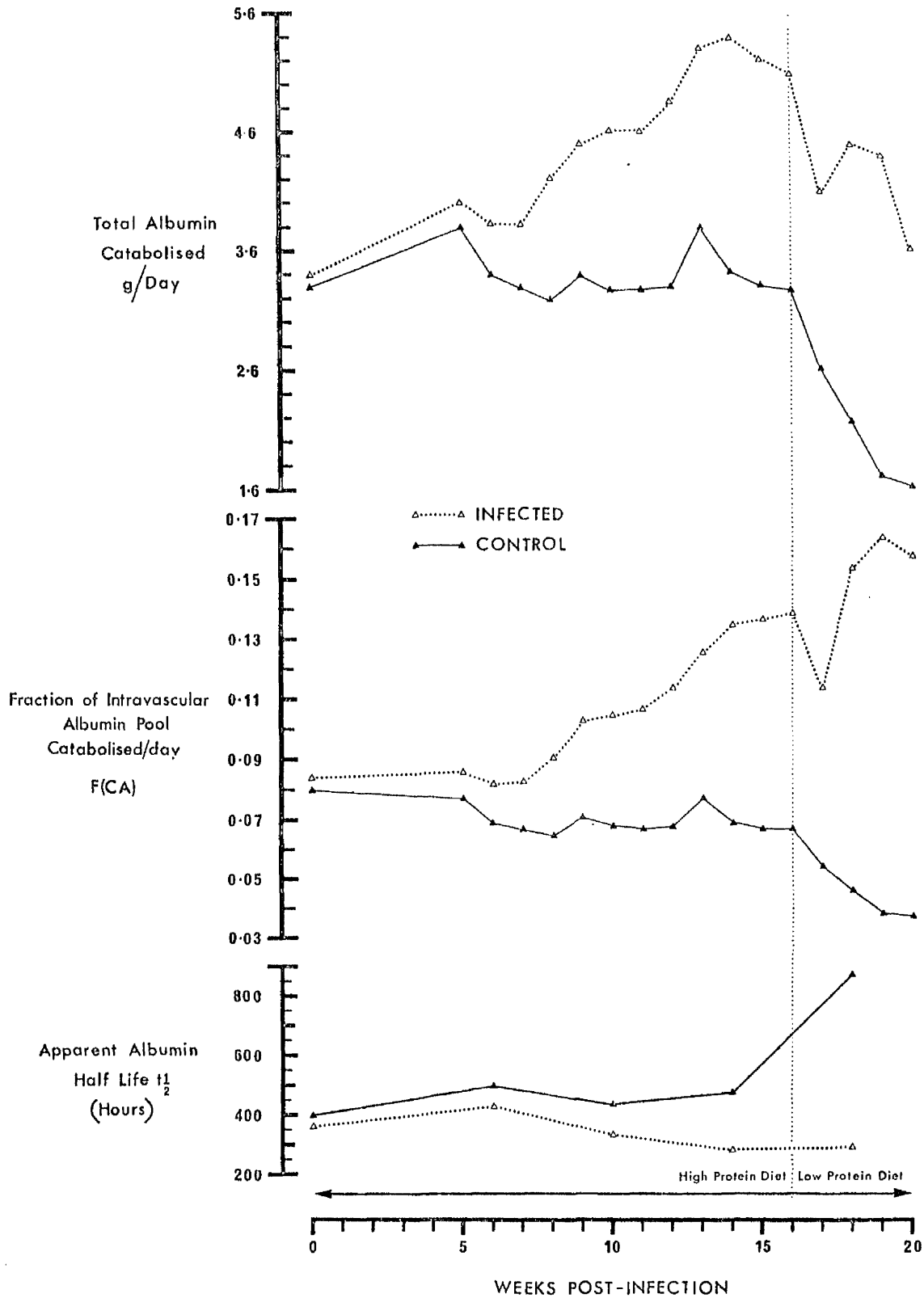


Fig. 9 : Effect of Diet on the Catabolism of Albumin in Fluke-Infected and Control Sheep.

values for the control sheep remained steady during this period although $t\frac{1}{2}$'s were somewhat lower and fractional and absolute rates of catabolism higher than recorded for the protein supplemented sheep used in the first experiment.

Transfer to the low protein diet did not affect the half-life of albumin in the infected sheep, nor to any significant extent their F(CA) values; but the amount of albumin catabolised fell sharply and by the 20th week had returned to pre-infection levels. By contrast, half-lives of the control animals lengthened considerably (from 20 to 36 days) and by the 20th week their fractional and absolute rates of catabolism were half those recorded at the outset.

As a reflection of their higher protein intake, albumin synthesis was significantly greater in these animals ($p < 0.001$) than in those of the previous experiment (Table 2). Synthesis was apparently unaffected by fluke migration, and was significantly higher than in the pair-fed controls during the subsequent 8 weeks of high protein feeding ($p < 0.05$). Since the total exchangeable albumin pool was not measured following the dietary change, albumin synthesis could not be estimated, but judging by the depletion which occurred intravascularly, this must have been substantially reduced in both groups.

Passage of Plasma and Albumin into the Gut

Losses of plasma and albumin into the gut, estimated from ^{51}Cr excretion in the faeces were similar and reasonably constant

in/

Table 2: Albumin Synthesis (mg/kg/day ± S.E.) in Sheep Infected with *F. hepatica* and Pair-fed Controls

	Control	Infected
-2 to 8 Weeks After Infection	123.7 ± 11.1	126.3 ± 7.0
8 to 16 Weeks After Infection	106.7 ± 3.9	135.5 ± 9.7

in all animals until the 7th week (about 50ml and 1.5g/day respectively), but increased subsequently in the infected group, reaching 150ml and 4g/day around week 16 (Fig. 10). Figures for the control sheep remained steady during this period. In the infected animals plasma losses were largely unaffected by the dietary change but fell by 60% in the controls to 15ml/day; albumin losses on the other hand dropped sharply in all animals and by the 20th week these were 2.2 and 0.8g/day in the infected and control groups respectively.

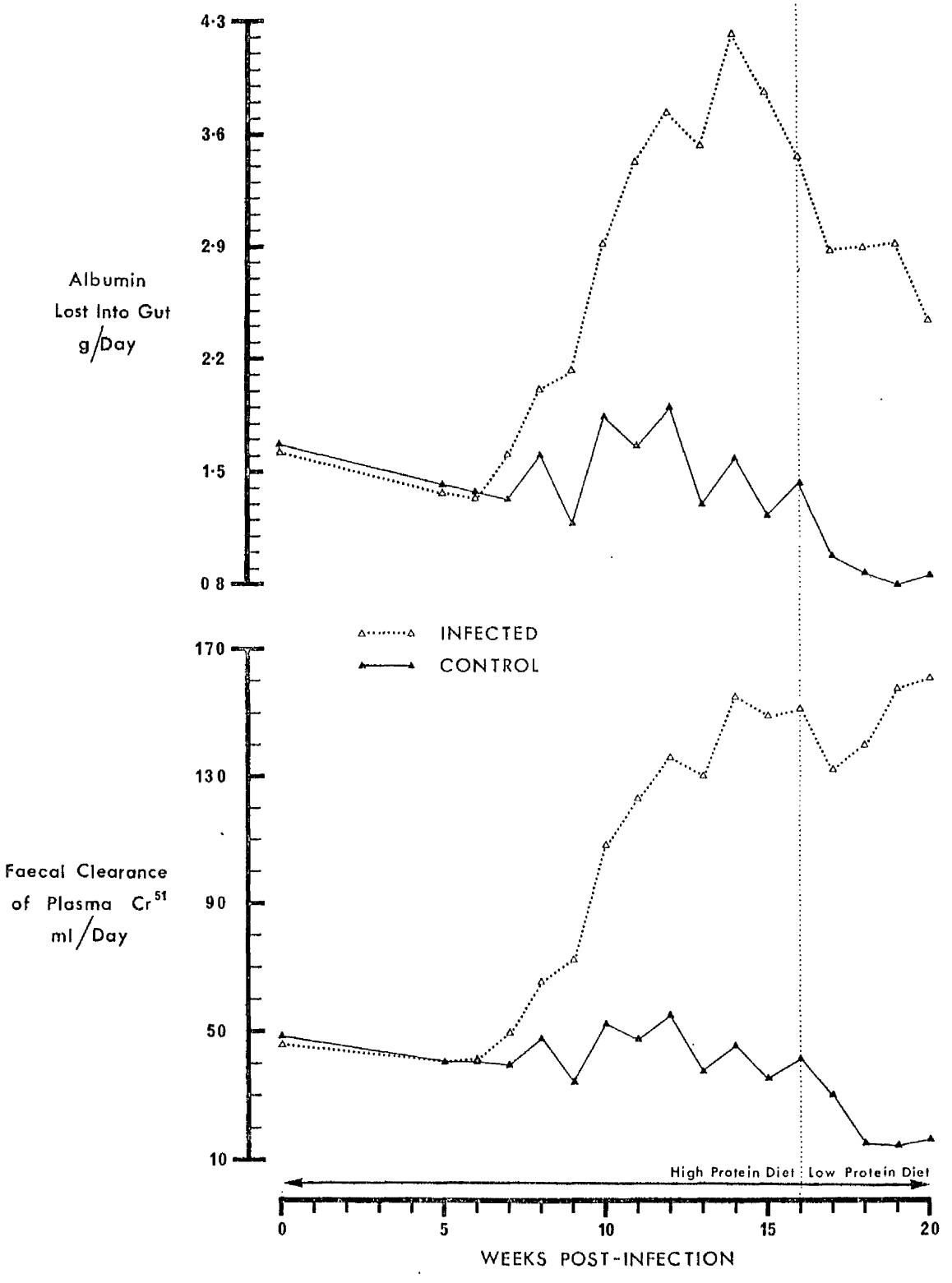


Fig. 10 : Effect of Diet on the Enteric Losses of Albumin and Plasma in Fluke-Infected and Control Sheep.

DISCUSSION

Previous studies on the hypoalbuminaemia associated with fascioliasis have stressed the importance of hypercatabolism and excessive protein loss into the gut during the biliary stage of the disease [13-18]. The present work confirms this general picture but illustrates the more complex nature of the different processes and inter-relationships involved.

The first aspect which merits discussion is the cause of the hypoalbuminaemia which developed in all infected animals during the course of fluke migration, i.e. the initial 7-8 weeks of infection. Over this period there was a fairly close correlation between the severity of the condition and both fluke numbers and protein intake; albumin levels deteriorating most in the sheep with the highest fluke burdens i.e. by 27% in experiment 1 (500 flukes) and 13% in experiment 2 (200 flukes), and within groups of similarly infected animals in those maintained on the lower protein diet i.e. by 36% as compared with 17% in association with average crude protein intakes of about 50 and 115g/day respectively.

In attempting to understand the aetiology of these changes a useful start may be made by considering their relationship to the accompanying changes in albumin pools. Two points are relevant in this connection. Firstly, in none of the infected animals was the intravascular pool of albumin depleted to the extent indicated by the drop in serum albumin concentration demonstrating that at least one of the factors involved in the early hypoalbuminaemia was plasma volume/

volume expansion. Indeed, in the first experiment this was the major factor in the group restricted to hay, being responsible for about two thirds of the drop in albumin concentrations, but was also an important feature in their better-fed counterparts, contributing about 30% during the first 7 weeks and 65% if the apparently more protracted fluke migration in these animals is taken into consideration and the results up to the 10th week included. Only in experiment 2 were elevations in plasma volumes not associated with marked hypoalbuminaemia. The second point about the changes in plasma albumin levels at this time, is that they were accompanied by roughly parallel, though again smaller reductions in the size of the total exchangeable pool. The significance of this finding is that since both the fractional and absolute rates of albumin degradation were the same as before infection, albumin synthesis must have been adversely affected, to a relatively greater extent in the heavier infections, and especially when these were superimposed on a low plane of nutrition; this is to some extent confirmed by the data in Table 1. The hypoalbuminaemia observed during the migratory phase of the infection would thus appear to result from increased plasma volumes and lowered rates of albumin synthesis.

In view of the fact that the migrating flukes inflict substantial damage to the liver during this period it is reasonable to assume that the destruction of albumin producing cells and changes in vasculature with the possible consequences of portal hypertension [33], are reflected in the reduced albumin synthesis and plasma volume expansion. However, a further feature of these infections which may have/

have some bearing on the above disturbances was the development of hyperproteinaemia, which significantly was much more pronounced in the first experiment, and within this experiment in the sheep restricted to hay.

Reduced plasma albumin levels in the presence of elevated globulin levels have been observed in a variety of clinical states and are considered by a number of workers to be the result of an extravascularly located regulatory system which acts by negative feedback to control albumin synthesis and thereby maintain the plasma oncotic pressure and volume (see review of Oratz [34]). While a rise in the intravascular colloid osmotic pressure due to hyperglobulinaemia, acting in concert with anaemia and perhaps portal hypertension may be the basis of the early increases in plasma volume observed in the heavy infections, it could hardly account for the attendant reductions in albumin synthesis since in all cases the depletion of body albumin was primarily from the extravascular compartment. Other workers have attributed the reciprocal relationship between plasma albumin and globulin levels to preferential utilisation of amino acids by cells involved in the production of globulins and other biologically important proteins [35]. Under such circumstances, direct cellular damage to the liver together with competition for available amino acids would be plausible causes of reduced albumin synthesis.

Perhaps the most interesting aspect of the investigation was the changes in albumin metabolism recorded following the parasites' entry into the bile ducts, and the way in which the various parameters were influenced by the hosts' nutritional status and fluke burden. Irrespective/

Irrespective of diet and fluke burden the essential features which distinguished the infected sheep from their respective controls were the high fractional and absolute rates of albumin catabolism and the equally dramatic losses of this protein as plasma into the gut of the parasitised animals. All these changes were first detected around the 6th-8th weeks of infection, and although their development and ultimate severity varied the resultant hypoalbuminaemia and depletion of albumin pools were much greater than recorded during fluke migration.

The enteric plasma losses were substantially more than the losses of whole blood described earlier (chapter 2), suggesting that in addition to the damage sustained by direct blood-sucking or indirect haemorrhage there was considerable seepage of protein through the biliary epithelium. This has been confirmed by Murray [19] who showed that the functional complexes of the biliary mucosa, which normally act as a seal around the epithelial cells, open up in the hyperplastic parasitised mucosa and thereby become hyperpermeable to macromolecules.

Although the daily plasma leakage was considerably more than could be expected from the loss of whole blood it was nevertheless found to reflect the numbers of flukes present within the host, with losses reaching about 0.7ml/fluke/day in mature infections irrespective of the host's plane of nutrition. Because of differences in plasma albumin levels, however, it was found that the enteric albumin losses were significantly larger in the animals on the higher planes of nutrition and in fact the sheep in the second experiment which harboured/

harboured the smaller fluke burdens experienced the largest enteric albumin losses. Similarly, while there was a marked positive correlation between the severity of the decline in serum albumin levels experienced by the different groups of infected sheep and the increased albumin catabolism when expressed in terms of the apparent half-lives and fractional catabolic rates, the situation was reversed when absolute rates of albumin catabolism were determined. For example, the amount of albumin catabolised by the infected sheep in experiment 2 with their smaller numbers of flukes was much higher than that observed in the better-fed infected sheep in experiment 1 which in turn was greater than the animals receiving hay alone.

These apparent anomalies are, however, primarily a result of the different dietary protein intakes experienced by the respective groups of animals. The reduction of albumin catabolism with decreasing dietary protein intake has been recorded by a number of workers [35-37] and is well exemplified by the control values in these present studies, the changes occurring in respect of both dietary quality and also quantity. Nevertheless the differences in total albumin catabolism between infected sheep and their pair-fed controls were larger in the animals on the higher planes of nutrition and since this feature was also associated with less severe reductions in total body albumin pools it must be concluded that the ability of infected sheep to replace lost albumin by synthesis was increased by higher protein intakes.

Indeed the estimations of albumin synthesis revealed that all infected animals produced more albumin over this period of infection compared/

compared to their control partners but the effect was more pronounced in those animals with the higher protein intakes. These findings imply that the liver has the capacity to recover from the damage inflicted during the migratory stage of the disease and to increase the production of albumin in an attempt to counteract the biliary loss of the protein. The ability of the infected animal to sustain this increased synthesis is improved by the provision of more protein in the diet.

SUMMARY

The distribution and metabolism of albumin in sheep infected with F. hepatica were studied using radioisotopic techniques in an attempt to establish the cause of the more rapid development of hypoalbuminaemia in infected sheep when their level of dietary protein intake was restricted.

Significant hypoalbuminaemia was observed only in the more heavily infected sheep (500 flukes) during the period of fluke migration and was found to result from a combination of plasma volume expansion and reduced albumin synthesis. The increase in plasma volumes, which developed earlier in the protein restricted sheep was possibly a reflection of the elevated serum globulin levels acting in concert with anaemia and portal hypertension. Reduced albumin synthesis, on the other hand, was considered most likely to result from direct cellular damage to the liver and possibly competition for available amino acids.

The most dramatic alterations to albumin metabolism occurred following the parasites entry into the bile ducts with all infected animals exhibiting marked hypercatabolism of albumin due to the loss of plasma into the alimentary tract. While the volume of the enteric plasma loss was proportional to the number of flukes present irrespective of dietary status, the results suggest that the ability of sheep to replace the albumin lost into the gut was increased by the provision of more protein in the diet.

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CHAPTER 4

The Relationship of Host nutrition and Fascioliasis: The aetiology of the body weight changes which develop in sheep following infection with F. hepatica.

INTRODUCTION

It is now generally accepted that F. hepatica infections adversely effect the host's productivity with the loss of body weight (or the reduction of liveweight gain) being one of the principle parameters on which this opinion is based. However, little information exists on the underlying factors responsible for this reduction in body weight, nor is it known which of the various body components contribute towards these losses, or if there are alterations in the relative proportions of the body constituents.

Fundamentally, for infected sheep to suffer a relative lowering of body weight compared to worm-free animals maintained under identical conditions, they would have to experience either a reduction in the amount of dietary constituents entering the body and/or an increased excretion of metabolites from the body. Reduced intake in the form of inappetence has been recorded in sheep infected with F. hepatica by a number of workers [1 - 4], and although it was invariably associated with emaciation there has been no detailed investigation carried out to assess its contribution to the changes in body weight. In order to examine this feature it is necessary to use a control system by which the adverse effects due to a reduction of food intake can be differentiated from the other metabolic disturbances of the disease. Such studies can be accomplished by a system of "paired feeding" which involves the pairing of an infected animal with a comparable worm-free control and restricting the food intake of the latter to the same level as its/

its partner. This system has been used successfully in the study of a number of parasitic diseases in which inappetence is a clinical feature [5 - 11], and although it was generally found that reduced feed intake was the primary cause of body weight reductions the fact that infected animals lost more weight than the pair-fed controls implied that other contributory factors were involved.

The results presented in Chapter one of this thesis were the first to provide extensive quantitative evidence not only of inappetence in fascioliasis but also the contribution made by this feature to the overall changes associated with the disease. It was found that of the sheep studied only those with fluke burdens in excess of 200 adult worms suffered any notable reduction of appetite and in common with other parasitic diseases this feature was found to be the principle factor, although not the only one, responsible for weight loss.

A further lowering of nutrient intake can be experienced if the digestion and/or absorption of the dietary constituents are adversely affected by the presence of the parasite. Although the liver fluke is only associated indirectly with the alimentary tract it is relevant to look briefly at digestive function in animals infected with gastrointestinal parasites such as Ostertagia, Haemonchus and Trichostrongylus spp. Despite the fact that animals infected with these parasites often suffer quite severe damage to areas of their gastrointestinal mucosa [12], the evidence for decreased digestion and/or absorption of the diet is far from conclusive. Many of the conflicting reports have been summarised in/

in reviews of Gibson [13], Reveron and Topps [14] and Symons [15]. The majority of the studies were based on the traditional balance technique in which the intake of a particular dietary constituent is compared to the amount excreted via the faeces. While this method is simple in principle it does not give a true measure of digestibility because some of the faecal material is of enteric rather than dietary origin. In the normal animal these enteric losses arise from gastric secretions and cells sloughed from the gut walls, and form only a small part of the faecal outputs. On the other hand, Ostertagia circumcincta [16], Haemonchus contortus [17] and Trichostrongylus colubriformis [8], have all been found to cause additional losses of blood constituents into the alimentary tract and therefore reduced digestibilities measured by the balance technique in animals infected with these parasites may be purely a reflection of increased enteric losses.

In more precise investigations carried out by Symons and his colleagues [18], using rats infected with Nippostrongylus brasiliensis the absorption of a number of amino acids, palmitic acid, bromsulphthalein and glucose was found to be inhibited in isolated loops of the parasitised jejunum. When, however, absorption from the small intestine as a whole was measured rather than the region affected by the parasite this same group of workers found no evidence of malabsorption [19, 20].

The fact that there is no convincing evidence for malabsorption in animals harbouring parasites actually within their gastrointestinal tracts reduces the likelihood of this feature being of significance

in/

in fascioliasis. One possible factor which can not be overlooked, however, is that the liver fluke interferes with the flow of bile (which plays an integral part in the digestive process particularly with respect to fats) either by physically obstructing the bile ducts or damaging the liver. Although jaundice which would be indicative of blocked bile ducts is a rare feature of fascioliasis, it is not uncommon for malabsorption of fat to be found in human patients suffering from liver disease with or without jaundice being manifest [21].

In common with the gastrointestinal parasites the available evidence in the literature of impaired digestive function associated with fascioliasis is conflicting. The digestibility of fat, protein and cellulose was found by Chubaryon [22] to be reduced in sheep 60 - 70 days after infection. Duwel and colleagues [23], reported the digestibility of crude fibre to be significantly reduced in lambs 12 weeks after infection although the values for other dietary constituents were comparable to those of controls. In other studies on sheep suffering from fascioliasis, normal digestibility of the diet was found by Peters and his co-workers [24] and could be calculated from the results of Sinclair [25]. Canale and colleagues [26], similarly found the digestibility of a number of dietary components to be unaffected in cattle infected with F. hepatica.

The enteric haemorrhage resulting from the feeding activities of the adult flukes within the biliary system is an obvious source of excessive losses of metabolites from the body. Although the loss of blood and plasma into the gastrointestinal tract has been quantified by a number of workers (see Chapters 2 and 3) the proportion of/

of the constituents which are irreversibly lost from the body has never been assessed, with the exception of haemoglobin iron, which, as discussed previously was found to be quantitatively excreted in the majority of animals studied. In view of the fact that the main bile duct empties into the anterior end of the alimentary tract it is unlikely that all the blood constituents will pass down the gut without some proportion being digested and reabsorbed. Indeed, most of the workers who have determined apparent digestibility coefficients in fluke-infected animals using the balance technique, have found values comparable to the controls which suggests that the enteric losses are completely reabsorbed.

Irrespective of the direct loss of metabolites in the faeces as a result of the biliary haemorrhage it was clearly shown in the preceeding chapters that the loss of blood constituents caused considerable disruption to the red cell and plasma protein metabolism of infected sheep. It is possible, therefore, that in an attempt to maintain normal physiological levels of the more essential haemoglobin and plasma proteins the fluke-infected animal will divert metabolites such as amino acids away from less important sites. The decrease in wool growth of sheep [27, 28] and milk yield of cattle [29, 30] following infection with F. hepatica are possible examples of such a mechanism. Further evidence is suggested by the results of studies involving other parasites. For instance, infection of laboratory animals with Nematospiroides dubius and Trichostrongylus colubriformis has been found to cause a depression of muscle protein synthesis [31] and also an increase in the catabolic rate/

rate of whole-body protein [32]. Increases in the urinary nitrogen losses of sheep infected with T. colubriformis [9] and O. circumcincta [33] and calves infected with Oesophagostomum spp. [34] could also be indicative of increased protein catabolism.

If such a mechanism is operative in parasitic diseases then clearly the quantity and quality of the host's diet will be of particular importance since the more nutrients available from the diet the less the animal will have to release from its body reserves. The observations on body weight recorded in the first chapter of this thesis revealed that animals infected with F. hepatica and fed only hay suffered earlier and more severe losses of liveweight than comparable animals given a high protein supplement. Indeed the poorer fed animals started losing weight compared to their pair-fed control partners even before the onset of biliary haemorrhage and this could indicate that such a low protein intake was insufficient to provide even the requirements for the immunological response without mobilising body reserves. The provision of a high protein supplement on the other hand enabled similarly infected sheep to maintain their body reserves until the system was stressed even further by the onset of biliary haemorrhage. Animals with much smaller fluke burdens and fed a high protein diet were found to gain weight at a similar rate to pair-fed control partners despite considerable blood loss and increased metabolic activity.

Body weight is not necessarily the most satisfactory parameter to use for assessing the overall effects of metabolic disturbances since all body components may not be affected to

the same extent. Indeed it is possible that a reduction in one body component may be accompanied by an increase in another. This latter feature was strongly suggested by the results presented in the first chapter of this thesis. Sheep infected with F. hepatica suffered less severe reductions in body weight than pair-fed controls during the terminal stages of the disease when feed intake was very low. It was during this period of infection that visible oedema was developing and hence it is reasonable to assume that water retention was masking further reductions in tissue mass in the parasitised animals. A comparable effect, only in reverse, has been noted by a number of workers studying human malnutrition (see review of Waterlow and colleagues [35]). In the early stages of treatment of patients with detectable oedema there is often a "lag phase" which may last several weeks, during which there is no weight gain in spite of good food intake and nitrogen retention.

Clearly a knowledge of the changes in body composition would provide a better understanding of the overall effect of Fasciola infection, and it was the aim of these present studies to examine the changes which occurred in two of the principle body components, namely protein and water. Nitrogen and water balances were determined, at intervals during the studies described in the previous chapters, by careful measurement and analysis of food and water intakes, and urine and faecal excretions.

In addition apparent digestibility coefficients of a number of dietary constituents were determined concurrently to establish to what extent impaired digestive function and/or increased loss of metabolites into the gut contributed to the overall changes associated with fascioliasis.

MATERIALS AND METHODS

Experimental Animals and Design

A detailed description of the animals and basic design of the two experiments was given in Chapter 1. Briefly in the first study groups of 8 Scottish Blackface wethers were placed on diets containing about 6% or 13% crude protein. Six weeks later 5 animals in each group were individually infected with 1,000 F. hepatica metacercairae, the remaining 3 worm-free sheep being paired on the basis of body weight and restricted to the same feed intake as an infected sheep. The animals were studied for a further 20 weeks or until the infected sheep were believed to be in extremis.

In the second experiment 6 Scottish Blackface wethers individually infected with 600 F. hepatica metacercariae together with 6 worm-free pair-fed controls were maintained on a high protein ration until 16 weeks after infection and then transferred to a low protein diet for a further 4 weeks.

At intervals during both studies, daily urine and faecal outputs and voluntary water intakes were measured over periods of 5 and 7 days duration in the first and second experiments respectively. Samples of feed, urine and faeces were taken for analysis in order to determine nitrogen and water balances, and the apparent digestibilities of various dietary constituents.

Collection of Urine and Faeces, and Sampling of Feedstuffs for Chemical Analyses

The individual faecal and urine outputs and feed intakes were/

were recorded daily during the balance periods and samples collected and prepared for analysis by the methods described earlier.

Chemical Analyses

Samples of feed and faeces collected in both experiments were analysed for dry matter and nitrogen content, and additional analyses for organic matter, ash and ether extractable material were performed in the first study. Urine samples were analysed for nitrogen and all the analytical methods used have been described in detail previously.

Calculation and Expression of Results

Nitrogen Balance

A large proportion of the nitrogen (N) present within an animal is incorporated into protein and since this element enters the body exclusively via the diet and is lost primarily through the urine and faeces then useful information on the changes in protein status can be obtained from the determination of N balance.

N balance = N intake - (Faecal N + Urine N)

The balance technique has received much criticism in recent years because in many studies N balances have not correlated particularly well with changes in body weight, a fact which is difficult to reconcile with the finding that on a fat-free basis the N content of the adult body is remarkably constant [36]. Wallace [36] discusses in some detail the errors inherent in the measurement of N balance and the possible causes of the apparent anomalies. The loss of N by/

by routes other than urine and faeces, such as sweat, gases released from the alimentary tract, skin and hair are difficult or impossible to measure during most investigations and since they usually amount to less than 1g N/day are generally ignored. Certainly losses of this magnitude are very small compared to the amounts of N excreted in the urine and faeces but in relation to the smaller N balance value they may represent a significant percentage.

In addition the errors in the measurement of feed intake and faecal and urinary excretion are cummulative, thus inevitable spillagesmean that N intake is overestimated and output underestimated. This feature which leads to erroneously high N retentions becomes more significant as the N intake increases.

A further problem of balance studies is the rate at which food passes down the alimentary tract i.e. the faecal material collected on any particular day is not derived from the feed eaten on the same day. This situation is further complicated in ruminants by the large volume of the rumen and its effect of mixing the contents of several days intake. For these reasons it is desirable in balance studies that food intake should remain steady; both with regard to amount and composition over the period of measurement, in order that excreta estimations (and faecal estimations in particular) are representative of dietary intake.

This point was taken into consideration in the design of these present studies whereby 7 days were allowed for the animals to become acclimatised to the metabolism cages before balance measurements were commended. Despite these precautions, the 5 day balance periods employed in the first experiment proved too short

to/

to overcome the normal irregularities of daily hay intake and the additional fluctuations in appetite caused by the parasite. It was therefore necessary to combine the data from 3 consecutive periods to obtain meaningful results. In the second study the results of two consecutive 7 day periods were combined in order to allow a more valid comparison between the two experiments.

Apparent Digestibility Coefficients

The apparent digestibility coefficients of the various dietary constituents analysed in each study were calculated as described earlier.

The values are only apparent measures of digestive efficiency because they do not take into account the faecal material which is not of dietary origin but arises from digestive secretions and cellular debris from the walls of the alimentary tract. No attempt was made to correct for these enteric losses in the present studies for two reasons. Firstly, because of paired feeding it is likely that the enteric losses arising from normal digestive function would be very similar in each pair of experimental animals. Secondly it would be pointless to correct for these relatively small enteric losses in the infected animals without similarly correcting for the much larger losses arising from the biliary haemorrhage.

Water Balance

In these present studies water balance was determined simply as:

water balance $\doteq$ (water drunk + food water) - (urine water + faecal water)

with no calculation of metabolic water (derived from the breakdown of fat, protein and carbohydrate within the body) and insensible water losses being made. These latter two parameters although of undoubted importance/

importance are difficult or impossible to measure directly. Methods for their indirect assessment have been devised [37] but their application to diseased animals was considered to be of doubtful value and unlikely to increase the validity of the limited information obtained from this simple model.

Water intake varies considerably among individual animals due to a number of factors such as genetic variation, the temperature of both drinking water and the environment, and food intake [38]. Many of the factors were either outwith the scope of the present studied or relatively constant for all sheep. However, information was available on the food intake and in order to lessen the individual variability of the water balance data all parameters were expressed in relation to dry matter intake.

RESULTS

EXPERIMENT 1

Two groups of 8 Scottish Blackface wethers were fed diets providing crude protein intakes of about 110g and 50g/day respectively. Six weeks after being introduced to the diets 5 animals in each group were infected with 1,000 F. hepatica metacercariae, the remaining sheep acting as "pair-fed" controls to infected partners. Nitrogen and water balances and digestibility coefficients were determined at intervals throughout the study, which lasted until the parasitised animals were in extremis i.e. 15 - 20 weeks after infection. The results presented in the text are the mean values recorded for the pair-fed animals. The results for all individual sheep are given in Appendix 4.

The changes in body weight of these sheep and their relationship to the crude protein intakes have been described in detail in Chapter 1 but because of their relevance to the data presented here the results are summarised in Fig. 1.

Nitrogen Balance

The results of the nitrogen balance determinations are presented in Tables 1 and 2. The pre-infection measurements made 1 week after the animals had been placed on their respective diets and before paired-feeding commenced showed that although nitrogen intake of the sheep receiving the compound diet supplement was about 8g/day more than those fed hay alone they were only able to retain about 1g/day more, because of a much larger urinary nitrogen excretion. Almost identical results were obtained for the 5 - 7 week/

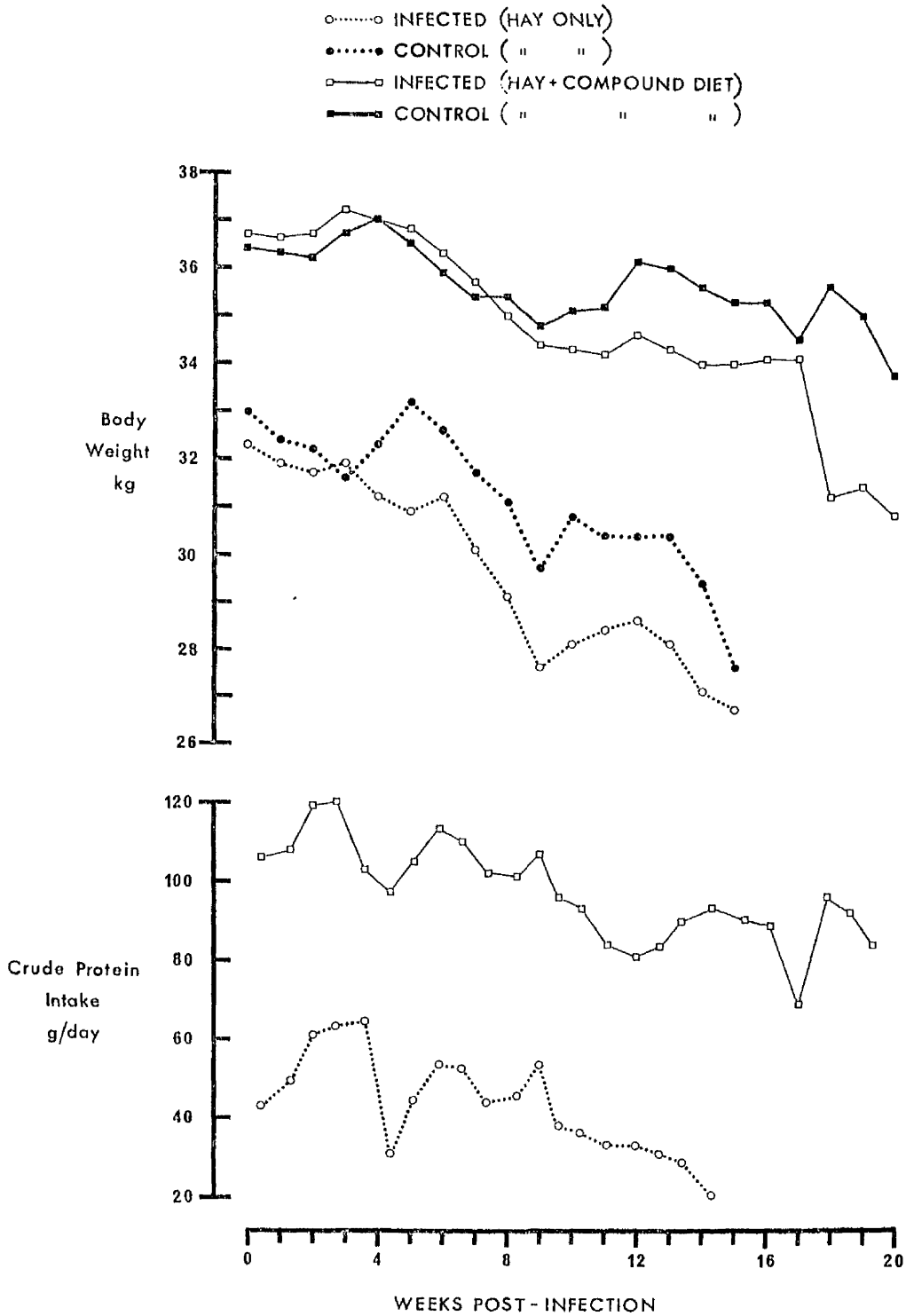

Fig. 1 : Body Weights and Crude Protein Intakes of Fluke-Infected and Control Sheep.

TABLE 1. Nitrogen Metabolism of Sheep Infected with *F. hepatica* and Pair-fed Controls Fed Hay Only

	Group	Nitrogen Intake (g/day ± S.E.)	Urinary Nitrogen (g/day ± S.E.)	Faecal Nitrogen (g/day ± S.E.)	Nitrogen Balance (g/day ± S.E.)
Pre-infection	Control	7.83 ± 0.31	1.58 ± 0.08	4.86 ± 0.18	+ 1.40 ± 0.22
	Infected	5.97 ± 0.39	1.60 ± 0.35	3.59 ± 0.21	+ 0.78 ± 0.22
5-7 Weeks After Infection	Control	7.51 ± 0.21	1.61 ± 0.24	4.21 ± 0.23	+ 1.69 ± 0.23
	Infected	7.53 ± 0.17	1.98 ± 0.28	4.43 ± 0.18	+ 1.12 ± 0.18
8-10 Weeks After Infection	Control	7.17 ± 0.68	3.36 ± 0.35	3.60 ± 0.25	+ 0.22 ± 0.44
	Infected	7.56 ± 0.67	4.70 ± 0.16	3.71 ± 0.41	- 0.84 ± 0.41
12-14 Weeks After Infection	Control	5.08 ± 0.28	1.59 ± 0.22	3.01 ± 0.27	+ 0.47 ± 0.22
	Infected	4.82 ± 0.36	3.06 ± 0.02	3.22 ± 0.14	- 1.46 ± 0.23

TABLE 2 Nitrogen Metabolism of Sheep Infected with *F. hepatica* and Pair-fed Controls Fed Hay and Compound Diet

	Group	Nitrogen Intake (g/day ± S.E.)	Urinary Nitrogen (g/day ± S.E.)	Faecal Nitrogen (g/day ± S.E.)	Nitrogen Balance (g/day ± S.E.)
Pre-infection	Control	16.13 ± 0.32	6.94 ± 0.32	6.43 ± 0.12	+ 2.76 ± 0.32
	Infected	14.45 ± 0.26	6.71 ± 0.32	5.90 ± 0.14	+ 1.84 ± 0.15
5-7 Weeks After Infection	Control	17.18 ± 0.73	8.15 ± 0.25	6.77 ± 0.65	+ 2.25 ± 0.22
	Infected	17.19 ± 0.70	7.89 ± 0.25	6.64 ± 0.46	+ 2.66 ± 0.48
8-10 Weeks After Infection	Control	16.01 ± 0.51	10.92 ± 0.59	5.59 ± 0.42	- 0.50 ± 0.49
	Infected	16.12 ± 0.53	11.71 ± 0.16	5.41 ± 0.15	- 1.00 ± 0.32
12-14 Weeks After Infection	Control	13.65 ± 1.71	7.46 ± 0.77	5.33 ± 0.67	+ 0.85 ± 0.43
	Infected	13.71 ± 1.68	8.51 ± 1.50	5.27 ± 0.55	- 0.07 ± 0.82
18-20 Weeks After Infection	Control	14.67 ± 1.96	7.26 ± 0.14	5.37 ± 0.88	+ 2.05 ± 0.93
	Infected	14.12 ± 2.06	8.38 ± 0.62	5.60 ± 0.39	+ 0.14 ± 1.04

week post-infection period with no differences being observed between control and infected groups on either plane of nutrition.

In the balance periods carried out after the 8th week of infection the parasitised animals exhibited progressively lower nitrogen balances compared to their pair-fed partners, and this was due to their excretion of larger quantities of nitrogen via the urine with faecal losses being very similar in both groups. In the case of the sheep fed hay alone the differences between the mean urinary loss experienced by the control and infected animals during the periods 8 - 10 and 12 - 14 weeks post-infection were significant ($p < 0.05$ and < 0.01, respectively) and lead to the parasitised sheep being in a mean negative balance of about 1 - 2g/day. Their pair-fed partners remained just positively balanced during these periods despite the nitrogen intake being reduced to about 5g/day as a result of the inappetence of the infected sheep.

The differences in the mean nitrogen balances of the sheep given the additional compound diet were not as pronounced as those recorded for their poorer fed counterparts during the same periods. Furthermore despite their much larger nitrogen intake the control and infected sheep had mean negative balances of 0.5g/day and 1.0g/day respectively between the 8th and 10th weeks after infection. It was during this period that all infected sheep suffered a reduction in their appetite for hay but the overall nitrogen intakes were little affected since some of the hay fed was of higher nitrogen content than that used throughout the rest of the experiment. Although the sheep were apparently well able to digest and absorb the nitrogen from the hay as shown by relatively normal faecal nitrogen losses, they were unable to retain it since urinary nitrogen losses were greatly increased in all animals.

Between/

Between the 12th and 14th weeks two of the better fed infected sheep had a reduced appetite for the compound diet, which lowered the mean nitrogen intake to 13.7g/day. Despite the reduced intake the controls had a positive balance of 0.9g/day but the infected sheep remained negatively balanced (-0.1g/day). The most seriously diseased animal in the high protein group died prior to the final balance period carried out between the 18th and 20th weeks after infection, but it can be seen from the results of the remaining sheep that the differences in mean nitrogen balances had increased to nearly 2g/day.

Apparent Digestibility Coefficients

The mean apparent digestibility coefficients are shown in Tables 3 and 4, and it can be seen that the presence of the parasite had little or no effect on the digestion and absorption of the various dietary constituents measured. The mean dry matter coefficients were comparable for both dietary groups throughout the study ranging from 62 - 66% and 58 - 65% in the low and high protein groups respectively. Similar results were found for the organic matter coefficients whose mean values ranged from 63 - 67% and 60 - 66% in the low and high protein groups respectively.

The ash digestibilities were more variable with the mean values of the control sheep fed the low and high protein diets ranging from 30 - 56% and 43 - 50% respectively. The mean values recorded for the infected animals were generally close to those of their control partners except in the final balance periods when they were somewhat lower, the difference being significant ($p < 0.02$) in the case of the sheep fed hay alone.

The/

TABLE 3. Apparent Digestibility Coefficients (% ± S.E.) in Sheep Infected with *F. hepatica* and Pair-fed Controls Fed Hay Only

	Group	Dry Matter	Organic Matter	Crude Protein	Ash	Ether Extract
Pre-infection	Control	62.7 ± 1.3	63.9 ± 1.6	37.9 ± 0.9	29.7 ± 2.4	60.2 ± 1.6
	Infected	64.8 ± 6.4	66.9 ± 6.4	39.9 ± 0.6	27.7 ± 8.5	63.9 ± 2.0
5-7 Weeks After Infection	Control	63.8 ± 2.2	65.6 ± 2.3	44.0 ± 2.9	35.3 ± 1.1	60.5 ± 1.6
	Infected	63.0 ± 2.0	64.7 ± 2.0	41.3 ± 2.3	33.6 ± 2.0	63.7 ± 2.3
8-10 Weeks After Infection	Control	62.3 ± 2.4	63.4 ± 2.3	49.6 ± 3.4	50.8 ± 4.7	61.3 ± 2.7
	Infected	63.2 ± 1.5	64.4 ± 1.7	51.2 ± 1.8	49.8 ± 2.3	59.6 ± 3.3
12-14 Weeks After Infection	Control	65.9 ± 1.2	66.6 ± 1.2	40.9 ± 3.5	55.6 ± 2.3	70.4 ± 2.3
	Infected	64.3 ± 1.7	65.4 ± 1.7	32.8 ± 4.6	47.5 ± 2.7	71.1 ± 4.7

TABLE 4. Apparent Digestibility Coefficients (% ± S.E.) in Sheep Infected with *F. hepatica* and Pair-fed Controls Fed Hay and Compound Diet

	Group	Dry Matter	Organic Matter	Crude Protein	Ash	Ether Extract
Pre-infection	Control	62.5 ± 1.6	64.1 ± 0.9	60.1 ± 0.7	43.3 ± 10.3	78.3 ± 2.5
	Infected	62.5 ± 0.9	64.1 ± 0.9	59.1 ± 1.6	44.0 ± 0.9	75.4 ± 0.4
5-7 Weeks After Infection	Control	61.5 ± 1.5	62.9 ± 1.7	60.7 ± 3.6	44.5 ± 6.7	79.0 ± 1.8
	Infected	62.4 ± 0.8	63.8 ± 0.8	61.5 ± 1.9	44.4 ± 3.9	78.3 ± 0.2
8-10 Weeks After Infection	Control	62.3 ± 2.3	63.6 ± 2.3	65.2 ± 2.6	49.4 ± 5.2	75.6 ± 0.7
	Infected	62.1 ± 2.7	63.5 ± 3.0	66.4 ± 0.6	49.1 ± 0.6	76.4 ± 1.2
12-14 Weeks After Infection	Control	63.7 ± 2.0	64.8 ± 2.1	60.9 ± 1.6	50.2 ± 5.7	80.5 ± 4.9
	Infected	64.6 ± 2.0	65.9 ± 1.7	61.3 ± 3.1	48.9 ± 5.1	76.4 ± 6.1
18-20 Weeks After Infection	Control	60.8 ± 1.7	61.9 ± 1.0	63.6 ± 1.2	48.4 ± 9.3	79.0 ± 5.0
	Infected	58.3 ± 0.6	60.0 ± 0.7	59.9 ± 3.1	38.4 ± 1.0	75.4 ± 6.0

The coefficients of crude protein digestibility reflected the dietary quality with the sheep fed the high protein supplement having mean values in excess of 60% while the animals restricted to hayhad mean values lower than 50%. Similarly during the 8 - 10 week period when a higher quality hay was fed, the apparent digestibilities were higher in all animals. During the final balance periods as in the case of the ash values the mean crude protein figures of the infected sheep were lower than their control partners although the differences were not statistically significant.

The ether extract values were unaffected by the parasite with mean coefficients generally higher in the better fed sheep (range 75 - 80%) than their poorer fed counterparts (range 60 - 71%).

Water Intake, Loss and Apparent Retention

The mean daily water intake (i.e. voluntary intake + food moisture), losses of water via the urine and faeces, and the resulting apparent water retention measured at intervals during the study are shown in Tables 5 and 6. There was considerable variation in the values recorded for individual animals despite their relation to dry matter intake, but nevertheless a number of interesting points were noted.

The mean water intake of the control sheep receiving the additional concentrates was consistently about 1ml/gD.M. greater than those fed hay alone. However, the greater water losses in both urine and faeces experienced by the former group meant that the apparent water retention was very similar in both groups (0.5 - 0.8 ml/gD.M.).

Following/

TABLE 5 Water Metabolism of Sheep Infected with *F. hepatica* and Pair-fed Controls Fed Hay Only

	Group	Water Intake (ml/gD.M. ± S.E.)	Urine Volume (ml/gD.M. ± S.E.)	Faecal Water (ml/gD.M. ± S.E.)	Water Balance (ml/gD.M. ± S.E.)
Pre-infection	Control	1.70 ± 0.05	0.41 ± 0.09	0.70 ± 0.04	+ 0.60 ± 0.08
	Infected	1.91 ± 0.29	0.46 ± 0.22	0.65 ± 0.10	+ 0.79 ± 0.09
5-7 Weeks After Infection	Control	1.76 ± 0.19	0.60 ± 0.17	0.63 ± 0.03	+ 0.53 ± 0.03
	Infected	2.45 ± 0.50	0.90 ± 0.35	0.73 ± 0.06	+ 0.81 ± 0.11
8-10 Weeks After Infection	Control	2.34 ± 0.26	0.95 ± 0.34	0.55 ± 0.04	+ 0.84 ± 0.05
	Infected	3.42 ± 0.78	1.77 ± 0.65	0.62 ± 0.03	+ 1.02 ± 0.19
12-14 Weeks After Infection	Control	1.83 ± 0.22	0.67 ± 0.24	0.67 ± 0.06	+ 0.49 ± 0.18
	Infected	2.88 ± 0.40	1.21 ± 0.37	0.83 ± 0.05	+ 0.85 ± 0.05

TABLE 6 Water Metabolism of Sheep Infected with F. hepatica and Pair-fed Controls Fed Hay and Compound Diet

	Group	Water Intake (ml/gD.M. ± S.E.)	Urine Volume (ml/gD.M. ± S.E.)	Faecal Water (ml/gD.M. ± S.E.)	Water Balance (ml/gD.M. ± S.E.)
Pre-infection	Control	2.61 ± 0.26	0.95 ± 0.13	0.92 ± 0.08	+ 0.74 ± 0.05
	Infected	2.58 ± 0.05	0.86 ± 0.07	0.87 ± 0.02	+ 0.86 ± 0.00
5-7 Weeks After Infection	Control	2.74 ± 0.21	1.01 ± 0.05	0.95 ± 0.11	+ 0.78 ± 0.08
	Infected	2.73 ± 0.16	0.89 ± 0.16	0.99 ± 0.03	+ 0.85 ± 0.03
8-10 Weeks After Infection	Control	3.53 ± 0.42	2.09 ± 0.31	0.74 ± 0.06	+ 0.70 ± 0.07
	Infected	3.36 ± 0.24	1.55 ± 0.27	0.77 ± 0.03	+ 1.05 ± 0.07
12-14 Weeks After Infection	Control	2.81 ± 0.31	1.31 ± 0.17	0.91 ± 0.12	+ 0.60 ± 0.08
	Infected	2.95 ± 0.20	1.10 ± 0.13	0.97 ± 0.06	+ 0.89 ± 0.06
18-20 Weeks After Infection	Control	2.47 ± 0.13	1.11 ± 0.32	0.85 ± 0.10	+ 0.52 ± 0.08
	Infected	3.90 ± 0.62	1.86 ± 0.71	1.14 ± 0.05	+ 0.90 ± 0.14

Following infection the water intake of the sheep fed hay alone increased to about 1 ml/gD.M. above that of their control partners, being apparent by the 5 - 7 weeks post-infection and persisting throughout the remainder of the study. Despite this increased intake, the apparent water retention was not markedly altered in infected animals because of increased urinary losses, and although their water retention was consistently some 0.20 - 0.35 ml/gD.M. greater than the controls following infection it was not statistically significant, and furthermore infected animals exhibited a larger water retention than the controls (0.2 ml/gD.M.) prior to infection.

Disturbances to the water metabolism of the infected sheep fed the better diet were not as obvious as those recorded for their poorer fed counterparts because of even greater variation between individuals particularly the controls. There was nevertheless a tendency for the majority of these infected animals to increase water intake and urinary excretion towards the terminal stages of the infection. The mean apparent water retention of the infected animals on this plane of nutrition was also consistently higher than their controls, with the largest differences (about 0.3ml/gD.M.) being recorded in the balance periods after the 8th week of infection.

EXPERIMENT 2

Six Scottish Blackface wethers were each given 600 F. hepatica metacercariae and paired to a worm-free control animal whose feed intake was restricted to the same level as its infected partner. During the first 16 weeks of infection the animals were maintained on/

on a high protein ration (13 - 15% crude protein) and for a period of 4 weeks thereafter on a low protein diet (8% crude protein). Balance periods of 14 days duration were performed throughout the study, with the exception of the period between the 2nd and 4th weeks of infection. Nitrogen and water balances, and apparent digestibility coefficients were determined during these periods. Only the mean values for the infected and control groups are presented in the figures but individual values are detailed in Appendix 4.

Because of their relevance to the data presented below, the results of body weight changes and crude protein intakes of these sheep, which were described in detail in Chapter 1, have been summarised in Fig. 2.

Nitrogen Balance

The nitrogen balance data recorded in this study are depicted in Fig. 3. The high quality of the diet used during the first 8 weeks of the infection lead to nitrogen intakes of 22g/day and positive balances of 4 - 5g/day in both infected and control animals. With the change to another high protein diet of slightly higher nitrogen content for the next 8 weeks, nitrogen intake increased to 27g/day but rather than retention increasing a larger quantity of nitrogen was excreted via the urine. Following the change to low protein feeding at 16 weeks post-infection nitrogen intake was reduced by a third and although urinary nitrogen losses were also greatly reduced all animals entered a negative balance in the order of 2 - 4g/day.

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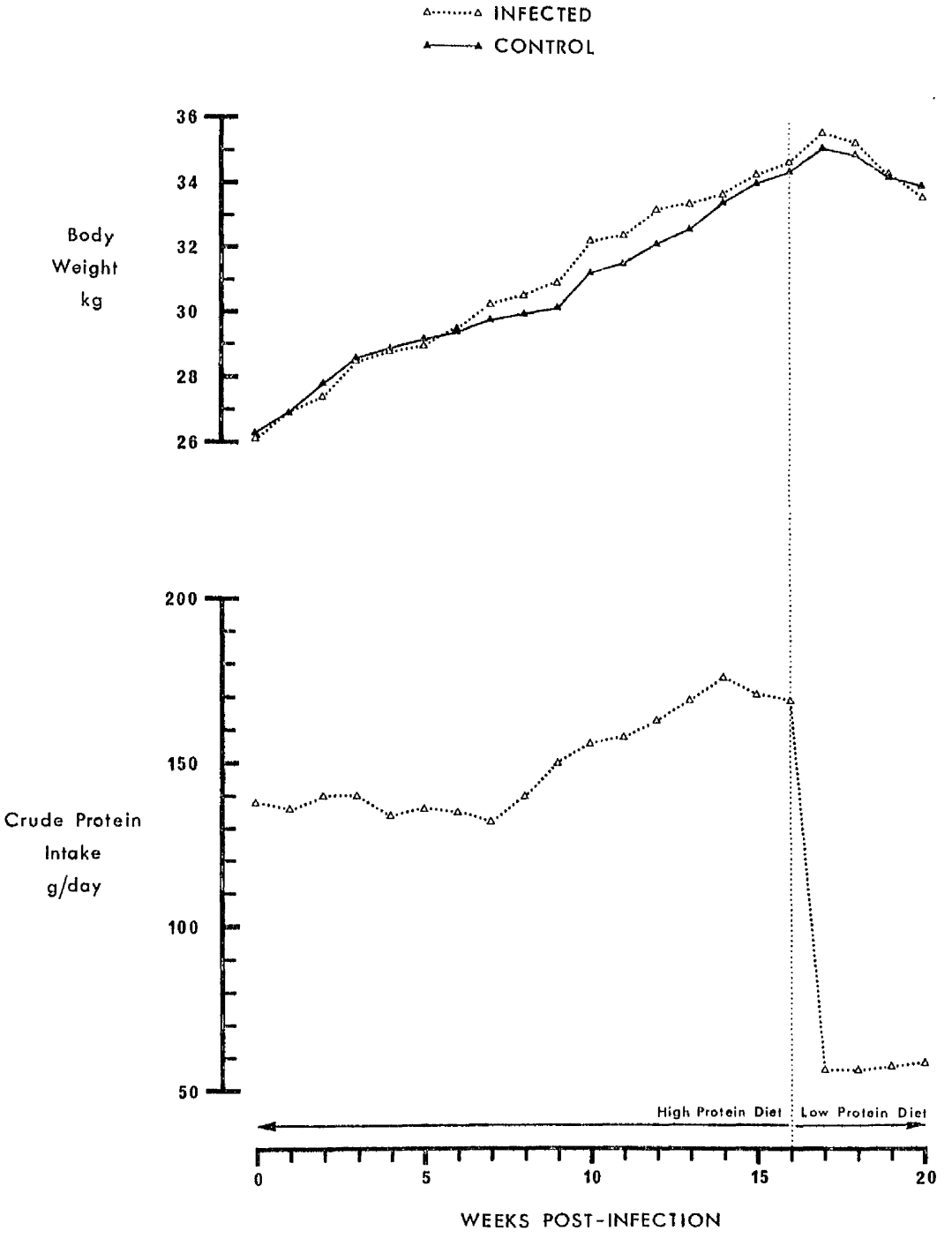

Fig. 2 : Body Weights and Crude Protein Intakes of Fluke-Infected and Control Sheep.

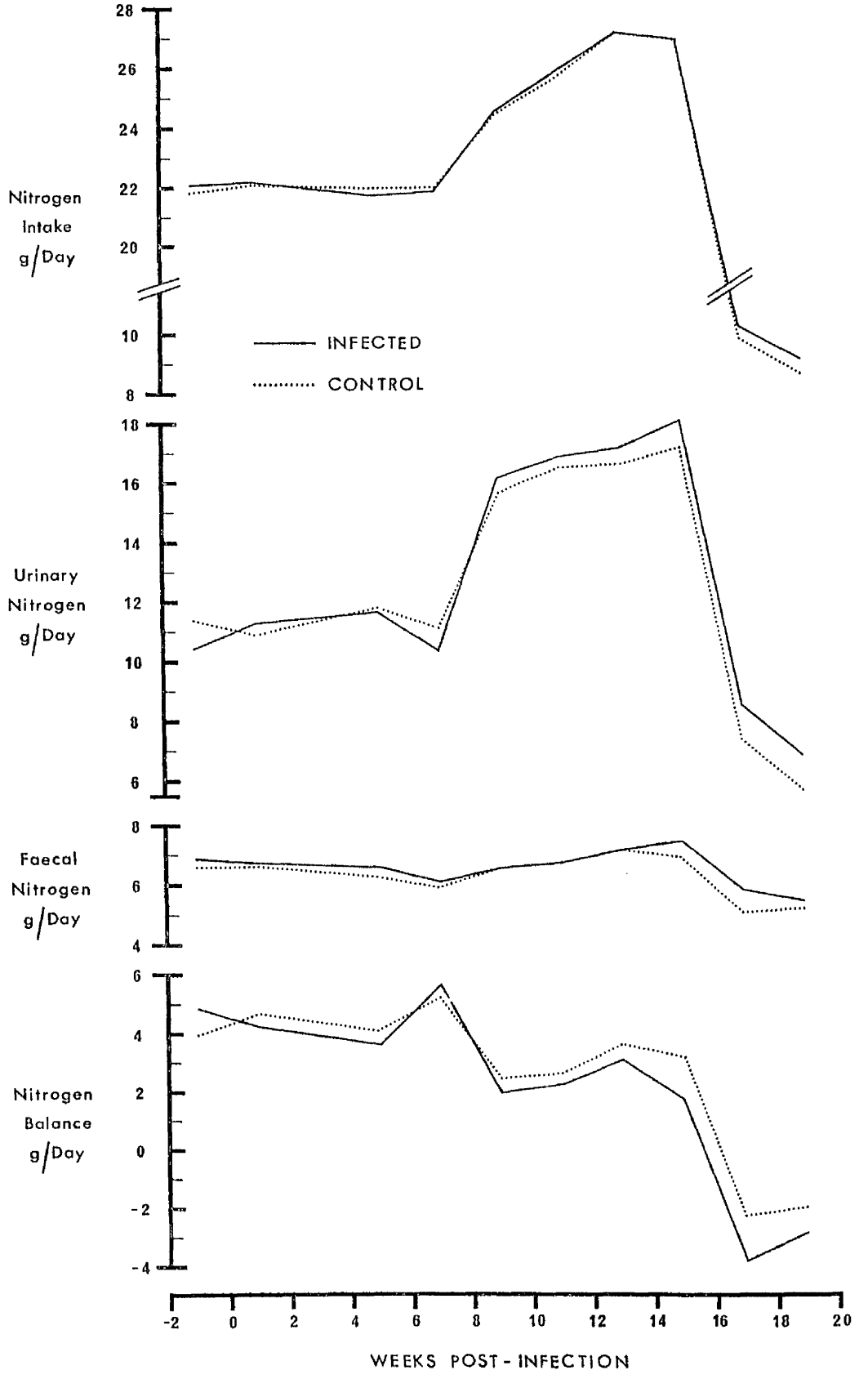

Fig. 3 : Nitrogen Metabolism of Fluke-Infected and Control Sheep.

At no stage of the study were there any significant differences in the nitrogen metabolism of infected and worm-free sheep although the amount of nitrogen retained by the infected animals was generally less than that of the controls. This latter feature became more apparent as the disease progressed with differences during some periods exceeding 1g/day.

Apparent Digestibility Coefficients

During the period of high protein feeding the digestibility of the dry matter component was relatively constant at 58 to 60% while the crude protein coefficient rose slightly from 70 - 74% (Fig. 4). The values of both components in the control and infected sheep were very similar throughout this period although the small difference of 1.8% recorded for the crude protein fraction between the 14th and 16th weeks post-infection was statistically significant ($p < 0.05$).

Following the change to the poorer quality ration the digestibilities of both components were markedly reduced in both groups of animals but to a greater extent in the infected sheep. Since, however, the range of values recorded for individual sheep was very large over this period the differences were not significant.

Water Intake and Apparent Water Retention

The mean water intakes and apparent water retentions recorded during this study are depicted in Fig. 5. During the period of high protein feeding the water intake of both groups increased progressively by about 1.45ml/gD.M.) with infected animals having

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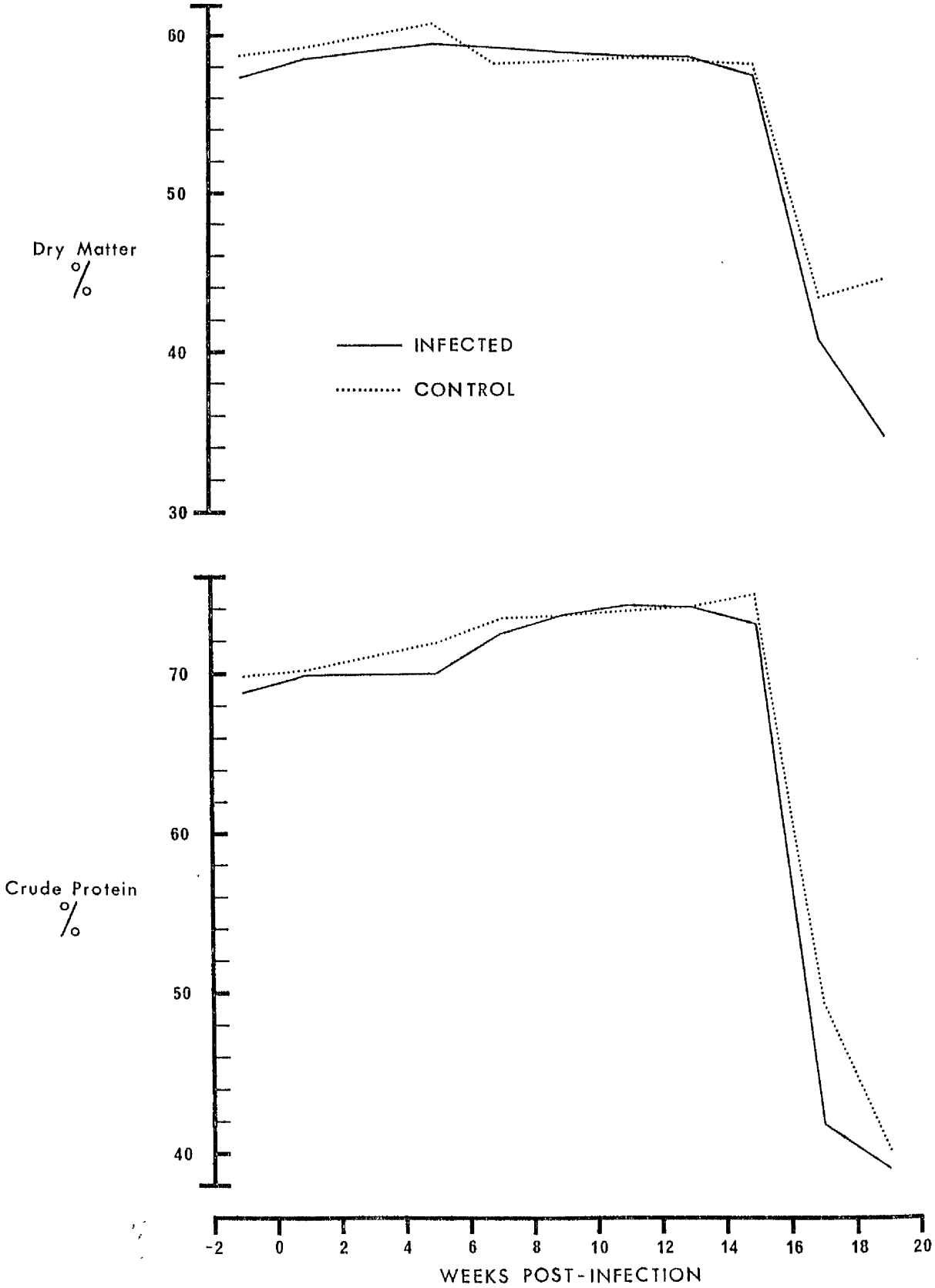

Fig. 4 : Apparent Digestibility Coefficients in Fluke-Infected and Control Sheep.

a mean intake consistently about 0.2ml/gD.M. greater than the controls. The apparent water retentions also increased slowly over this period in both groups of sheep but while the values were very similar during the first two periods from the 4th week of infection onwards the parasitised animals retained more water than the controls. The difference was only in the order of 0.1 - 0.2ml/gD.M. but between the 4th and 8th weeks it was significant ($p < 0.05$).

Following the change to the low protein diet the water intake of the controls decreased, but in contrast the value for the infected sheep remained high. The exceptionally large mean intake of the infected sheep between the 16th and 18th weeks of infection was due primarily to a very high value in one sheep. Despite the relatively larger water intake of infected sheep during this period water balance was not markedly affected and in fact during the final balance period the values in both groups were comparable.

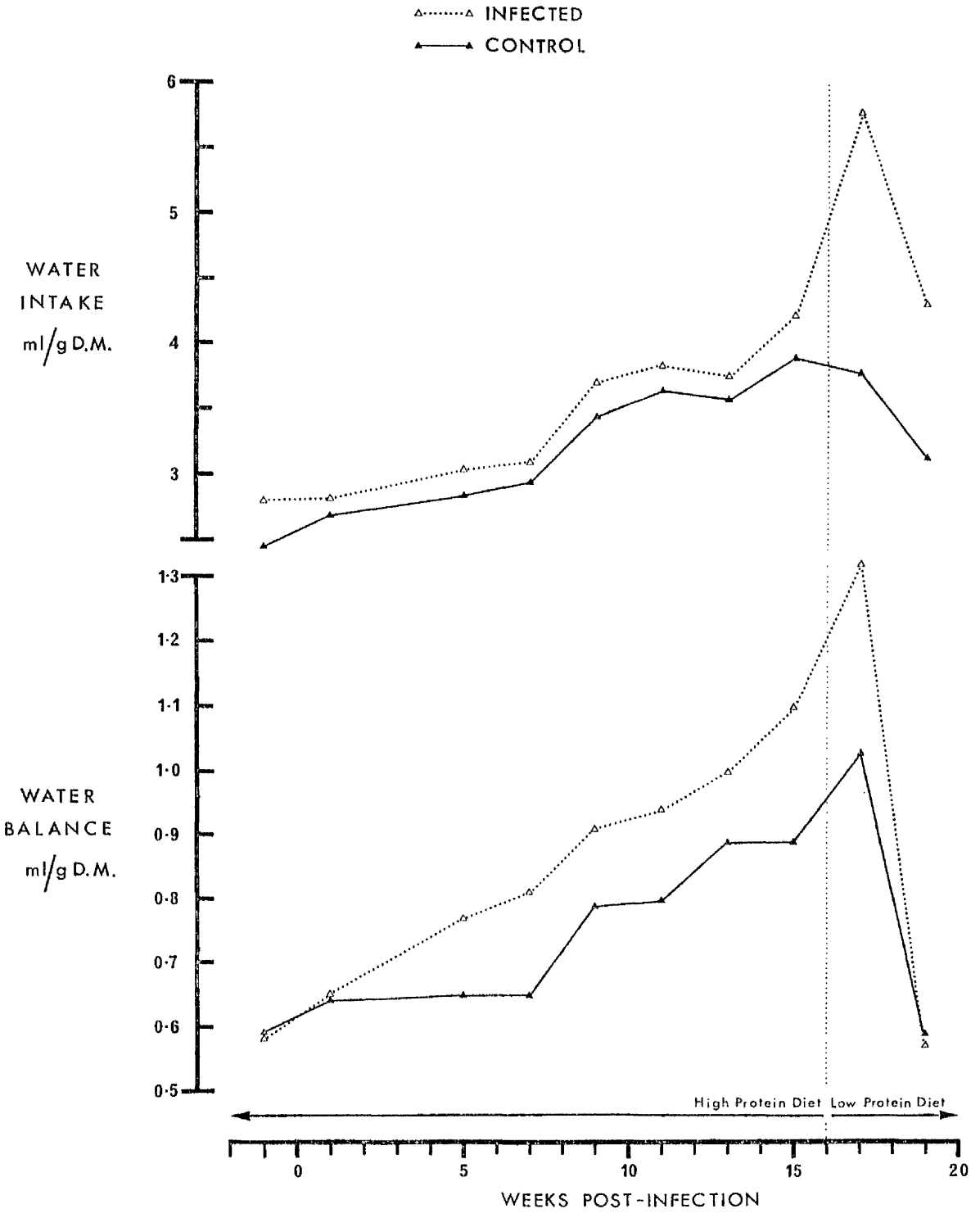

Fig. 5 : Water Intake and Balance of Fluke-Infected and Control Sheep.

DISCUSSION

In an earlier chapter of this thesis, sheep infected with 500 F. hepatica were found to lose more weight than worm-free sheep maintained at a similar level of dietary intake. During the studies described in the present chapter the possible mechanisms involved in this weight loss were investigated by monitoring the changes in two of the principle body components, namely protein and water.

Nitrogen balance studies have shown that the weight loss of fluke infected sheep, compared to pair-fed controls, may be explained at least in part by a depletion in the nitrogen and hence the protein content of the diseased sheep. The increased excretion of nitrogen by the diseased animals, which became apparent only after the onset of biliary haemorrhage, occurred via the urine and developed more rapidly in the sheep on the poorest plane of nutrition. Little difference was found in the nitrogen metabolism of sheep harbouring smaller numbers of flukes (i.e. 200) compared to their pair-fed controls even when the animals were changed from high to low protein feeding.

Before discussing the interpretation of the nitrogen balance data presented in this chapter certain anomalies should be noted. In particular there was little correlation between the nitrogen balance values and the changes in the body weight. For example, during the first experiment the control animals fed only hay steadily lost weight, although a positive nitrogen retention was recorded for each balance period. In contrast, the control animals that/

that received the additional compound diet were in negative balance between the 8th and 10th weeks after infection when they actually gained weight but during the final balance period (i.e. 18 - 20 weeks after infection) these sheep were in positive balance while suffering their most severe reduction in body weight.

Although these anomalies may be attributed to changes in the body content of fat or fluctuations in the volume of material in the alimentary tract it would have been reasonable to expect a more direct correlation between nitrogen balance and body weight, than that actually observed. A positive nitrogen retention in sheep which are losing weight may be attributed to errors of the technique since, the unmeasured loss of nitrogen by routes other than urine and faeces as well as the cummulative errors of spillage will all tend to result in an overestimation of the balance [36]. A negative nitrogen balance in an animal that is gaining weight cannot be explained in this way but may reflect urine and faecal outputs which were unrepresentative of the dietary intake during the balance period, despite the attempts to minimise this feature.

For these reasons it was not considered valid to interpret the nitrogen balances directly as quantitative measures of body protein changes. However, the use of paired feeding in these studies meant that the daily dietary fluctuations and also the sources of error associated with the technique would have been very similar in both the control and infected sheep. Consequently it is reasonable to assume that the differences in the nitrogen data recorded in the infected sheep and their control partners provide/

provide a good assessment of the changes in body protein resulting from factors other than inappetence which accompany infection with F. hepatica.

On this basis it is clear from the results that sheep harbouring 500 flukes retained less nitrogen than their pair-fed controls. The interesting feature about the lower nitrogen retentions of the infected sheep was that, although they were observed only after the onset of biliary haemorrhage, they resulted from the excretion of increased amounts of nitrogen in the urine but not in the faeces. Greater faecal nitrogen excretion might have been expected from the large quantity of endogenous protein which was lost into the gut during the course of the disease (Chapter 3).

Increased urinary nitrogen outputs have also been observed in sheep infected with T. colubriformis [9], H. contortus [39], O. circumcincta [33] and S. mattheei [40], but whether the physiological changes responsible for this feature are the same in each host-parasite system has yet to be established. The most detailed investigations into the nitrogen metabolism of parasitised sheep has been carried out by Roseby [9] who studied T. colubriformis infections. This worker found that the increased urinary nitrogen losses were due to an elevation in urea excretion with the losses of other nitrogenous compounds remaining unaltered. He also found that there was a change in the site of protein digestion and absorption within the alimentary tract being shown by a reduction in the amino acid nitrogen absorption from the small intestine of infected sheep and a corresponding increase in the absorption/

absorption of ammonia from the large intestine [41]. From these findings he concluded that little of this ammonia absorbed from the large intestine was used in amino acid synthesis but rather was converted to urea and excreted. Whether the presence of other parasites, particularly those situated in organs other than the gastrointestinal tract, also cause changes in the site of protein digestion has to be elucidated.

In common with F. hepatica the other parasitic infections which have been found to cause increased urinary nitrogen excretion also cause increased leakage of blood or plasma into the lumen of the gut [8, 16, 17, 42]. In these present studies both features appeared to be closely correlated since the lower nitrogen retentions of infected sheep compared to their control partners became apparent only after the onset of biliary haemorrhage and furthermore, the difference in the nitrogen balances increased as the volume of blood lost increased. The plasma proteins form part of the labile nitrogen pool of the body and the excessive loss of these materials into the gut with subsequent breakdown and reabsorption represents an increased turnover of this important protein pool. Parkins and his colleagues [33] considered that the elevated plasma urea levels found in sheep infected with O. circumcincta could reflect an increased turnover of this labile pool caused by an enteric plasma leakage. Indeed a change in the turnover rate of these proteins could explain why the excessive nitrogen loss developed more rapidly in the animals on the poorer plane of nutrition compared to their better fed counterparts. In the preceeding chapter it was shown clearly that the rate of albumin turnover

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was correlated with the level of protein feeding, i.e. turnover increased as protein intake increased. In sheep on two planes of dietary protein intake and therefore with different levels of protein turnover, the loss of similar quantities of blood proteins into the gut will result in a relatively larger increase in protein turnover in the animals on the lower protein diet. Using the same argument it is perhaps not surprising that little difference was recorded in the nitrogen balances of infected and control sheep in the second experiment during the period of high protein feeding. However, on the same basis it would have been expected that the difference in the nitrogen balances of these animals following the change to the low protein diet, would have been more marked than that actually observed.

Another theory for the increased excretion of nitrogen following infection with F. hepatica is the excessive catabolism of tissue and in particular muscle protein. While it is difficult to envisage a mechanism by which this parasite would cause a direct breakdown of the tissues, the passage of large amounts of blood or plasma into the gut will almost certainly increase the amino acid requirement of organs such as the liver and bone marrow that manufacture the blood proteins. The essential requirement of haemoglobin and albumin for normal body function may result in the diversion of amino acids away from less essential tissues such as the skin and muscle. The likelihood of such a mechanism being operative in host-parasite systems is clearly suggested by the work of Symons and Jones [31, 32] who found that the incorporation of ^{14}C-L-leucine into the skeletal muscle protein was depressed while incorporation into/

into liver protein was elevated in laboratory animals with nematode infections of the small intestine. While it is considered that increased protein degradation in the large intestine and/or an increased turnover of the labile nitrogen pool are directly responsible for the excessive loss of nitrogen in parasitised animals it is likely that these losses are reflected to a large extent by a reduction in the lean tissue mass, which is depleted in order to provide the amino acids necessary to maintain the more essential body proteins.

If lean tissue is defined as a fat-free non-body tissue containing 25% protein and 75% water then the degradation of 1kg of lean tissue will yield 40g of nitrogen (since 1g N is equivalent to 6.25g of protein). The more heavily infected sheep in these present studies were losing on average about 1.5g N/day more than their control partners over a period of about 8 weeks, which represents approximately 2kg of lean tissue. While losses of this magnitude would have contributed substantially to the differences in the body weights of the infected and control sheep, they do not explain completely the changes in body weight. For example, during the 2 to 3 weeks preceeding death when the largest differences in nitrogen balance were observed; the body weights of infected and control sheep began to converge. Also the difference in the body weights of the infected and control sheep fed only hay which became apparent prior to the onset of biliary haemorrhage can not be attributed to a loss of protein since the nitrogen balances of the two groups determined during this period were comparable.

Water/

Water is the largest single body component making up about 60% of a well nourished sheep [43] and disturbances to fluid distribution and/or content following infection with F. hepatica are reflected in the clinical appearance of oedema and ascites. Attempts to monitor the changes in body water content using the conventional balance technique was never expected to provide particularly accurate reflections of the changes in body water since in addition to the technical errors inherent in any balance study, sizeable gains and losses to body water take place, the measurement of which were outwith the scope of this present study. Although insensible water losses and metabolic water gains have been predicted with reasonable accuracy for normal animals using indirect techniques [37], the application of such criteria to diseased animals must be open to question since many of the physiological factors on which they rely e.g. respiratory rate, body temperature and body composition, will almost certainly be affected by the disease.

The most interesting feature to emerge from the water balance data was the increase in water intake relative to dry matter intake of the majority of the infected animals. This phenomenon which became apparent at a relatively early stage of the infection (i.e. 4 - 5 weeks after infection) has not previously been reported in connection with fascioliasis, and its physiological significance is at present obscure. Despite the increased water intake, the parasitised sheep exhibited only marginally larger water balances than their control partners because of increased urinary flow. It must be stressed, however, that a positive water balance of only 0.1ml/gD.M. in the sheep in these present studies would have led

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to a retention of about 500 - 700ml water/week. Clearly even such a small balance sustained over a number of weeks would have had a marked effect upon body weight and composition.

Over the last 30 to 40 years a number of techniques based on the dilution principle and using inert or radioactive markers have been developed, to measure the size of the body fluid spaces and so provide information on the body composition of the living animal [43]. Indeed a number of workers have successfully used tritiated water to predict not only the total body water but also the weights of fat, protein, ash and energy present in normal sheep [44, 45]. At intervals during these present studies attempts were made to measure total body water and extracellular fluid spaces using tritiated water and ^{24}sodium respectively. The results, which did indicate an expansion of both compartments in the more heavily infected sheep compared to their control partners, were however too inconsistent to merit inclusion in the thesis.

The apparent digestibility coefficients determined in these present studies revealed that F. hepatica had little or no detrimental effect on the overall digestive function of sheep, in fact the similarity of the values in control and infected sheep would indicate that the latter were able to completely digest and reabsorb the blood consistuents lost through biliary haemorrhage. However, in view of the work by Roseby [41], which was mentioned earlier, it is possible that the sites of digestion within the alimentary tracts of infected sheep may be altered, with a consequential change in the relative proportions of the products of digestion which are eventually absorbed into the body.

SUMMARY

Nitrogen and water balances were determined in fluke-infected and pair-fed control sheep, in an attempt to gain an insight into the body weight changes recorded following infection with F. hepatica.

The more severe reductions in the body weights of sheep harbouring 500 flukes compared to their pair-fed control partners was found to be due in part to a loss of protein. Reduced nitrogen balances were recorded in the infected sheep only after the flukes had entered the bile ducts and the excessive nitrogen excretion occurred via the urine and not in the faeces as might have been expected from the loss of blood constituents into the alimentary tract. The differences in the nitrogen retention of infected and control sheep increased as the disease progressed and also developed more rapidly when the animals were fed a poorer quality diet.

The majority of infected sheep drank more water following infection and although they also excreted larger volumes of urine there was a tendency for increased retention of water particularly during the later stages of the disease.

Body weight changes in sheep harbouring 200 flukes were similar to those observed in their pair-fed controls and although the nitrogen and water retentions were never markedly different it may be significant from the point of view of body composition that the nitrogen balance was consistently lower and the water balance consistently higher in the infected sheep compared to the controls.

The determination of the apparent digestibility coefficients of a number of dietary constituents revealed that F. hepatica had little or no effect on the overall digestive function of sheep.

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CHAPTER 5

Observations on the Pathophysiological Changes Occurring in Sheep Exposed to Different Levels and Duration of Infection with S. mattheei

INTRODUCTION

Schistosomiasis has long been recognised as a common infection of African domestic livestock, with S. mattheei predominating in Southern and Central regions and S. bovis in Northern and Eastern areas (see review by Hussein, [1]), but only recently has the economic importance of the disease become recognised and its pathogenesis investigated in any detail. The pathogenesis of natural and experimental infections has been attributed partly to the presence of dead worms, but mainly to the eggs and the granulomatous reactions which they elicit within the tissues and vasculature of affected organs, particularly the alimentary tract, liver and lungs [2-5]. The functional significance of the hepatic and pulmonary lesions is obscure in domestic animal schistosomiasis but the importance of the numerous eggs in the mucosa and submucosa of the small and large intestines is clear: haemorrhage caused by the passage of eggs into the faeces is clearly reflected in the clinical features of the disease, including anaemia, hypoalbuminaemia, inappetence, unthriftiness and a haemorrhagic diarrhoea [2, 6, 7]. It is also reflected in the pathophysiology of the disease, which at least in the case of acute and subacute S. mattheei infections in sheep includes losses of substantial amounts of red cells and albumin into the gut and excretion of a large proportion of their breakdown products in the faeces, impaired digestion and absorption of a number of dietary constituents, haemodilution, and for some as yet unexplained reason, a failure to regenerate red cells lost through haemorrhage [8-12]. The onset of all these changes coincides with the appearance/

appearance of eggs in the faeces and deaths often occur when faecal egg counts are maximal, i.e. between the 3rd and 5th months of infection [7, 13].

While there is no doubt that outbreaks of acute disease and the accompanying mortalities have helped to focus attention on the veterinary importance of this parasite, and that infections of this type are likely to become more prevalent through improved methods of husbandry and water conservation, it is probable that the greatest economic losses arise from the insidious long-term effects of more modest infestations. However, although the prevalence of the infection is both high and widespread [14], apart from the work of McKenzie and Grainge [15] demonstrating poor growth rates in animals infected with 1,000 cercariae there is little evidence that sub-lethal infections have any long-term adverse effects on the clinical condition or physiology of sheep.

There is also remarkably little known about the host-parasite relationship itself and how this might change during longstanding infections. For instance, the observation that animals which survive the acute haemorrhagic stage of the disease subsequently show signs of recovery [11] could be indicative of a reduction in worm numbers and/or their fecundity as the duration of the infection increased. However, although a slow decrease in faecal egg counts does occur in sheep after 5 months, this is not apparently accompanied by elimination of the parasite [13].

Since S. mattheei is of considerable veterinary importance, there is an obvious need for a clearer definition of the disease itself/

itself and its relationship to the parasites' numbers and reproductive activities. The present study was therefore designed with a view to comparing the host-parasite relationship during the course of acute and longstanding chronic infections and hence assessing more accurately their likely economic impact. For this purpose sheep were infected with 10,000 or 5,000 S. mattheei cercariae, and together with uninfected controls studied over a period of 12 months using clinical and radioisotopic methods. A number of animals were necropsied periodically to assess the intensity of infection in terms of worm numbers, faecal and tissue egg counts.

MATERIALS AND METHODS

Experimental Animals and Design

Forty 1 year-old Suffolk X Border Leicester wethers were purchased commercially and after treatment to remove gastrointestinal parasites maintained under conditions which precluded further infection. S. mattheei cercariae were collected from snails infected with miracidia obtained from hamsters. Eleven sheep were each exposed percutaneously to 10,000 cercariae, and a further 16 animals to 5,000 cercariae; the remainder acted as worm-free controls. Throughout the study, which continued until the 57th week of infection,the animals were given free access to water and except during periods of confinement in metabolism cages when the diet consisted entirely of a high protein compound ration, the animals were fed hay supplemented with a commercial concentrate ration.

Blood samples were taken weekly for PCV determination. Serum samples were collected weekly during the first 30 weeks and thereafter fortnightly for protein analyses, and the animals were weighed and samples from the rectum obtained at similar intervals for faecal egg counts. At intervals, 4-8 animals from each group were placed in metabolism cages for periods of 2-3 weeks to monitor albumin and red cell turnover; during these periods 10 ml 0.75% KI was given orally each day. The animals were necropsied at 13, 35 or 57 weeks after infection, the worms recovered by perfusion and various tissues collected for the determination of egg counts.

Haematological and Biochemical Determinations

Measurements were made of PCV, total serum proteins, serum albumin and globulin concentrations as described previously.

Parasitological Measurements

The techniques used to recover adult schistosomes and count the eggs in tissues and faeces have already been described.

Blood Volumes, Red Cell and Albumin Turnover Measurements

Plasma volumes (Vp) were measured by application of the dilution principle using ^{125}I-labelled albumin and circulating red cell volumes (RCV) by injecting a suspension of autologous erythrocytes labelled with ^{51}Cr and collecting a blood sample 15 mins later. The radioactivity of a 1 ml aliquot of this sample corrected for venous haematocrit was divided into the injected activity to obtain RCV. The simultaneous measurement of Vp and RCV by these methods provides a reliable measure of blood volume (BV) and when related to body weight allows comparison between animals of different weight.

Erythrocytes labelled with ^{51}Cr also provide a useful index of red cell survival based on the half-life ($t\frac{1}{2}$) of these cells in the circulation. This was measured in the present studies by converting the radioactivity of each blood sample taken daily after injection to activity/ml packed red cells and expressing this as a percentage of the 15 min post-injection value. From a semi-logarithmic plot of activity against time the half-life was calculated as the time taken for the activity to fall by 50%. The normal lifespan of the red cell in sheep is about 120 days [16] and since ^{51}Cr red cell $t\frac{1}{2}$ values are usually around 15 days it is obvious that the latter grossly underestimate true red cell survival. The reason for this is that the disappearance of labelled cells from the circulation reflects two main processes - loss of red cells by senescence or breakdown/

breakdown and elution of the isotope from intact cells [17, 18]. Although elution is very much more marked during the first few days following injection of labelled cells (over this period more than 60% of the injected activity may be lost), the process continues as long as any labelled cells remain in the circulation, hence the prefix "apparent" when referring to red cell half-life values measured with ^{51}Cr.

Plasma iron turnover rates and loss of blood into the gut were estimated using ^{59}ferric citrate, and albumin pools, catabolic rate and enteric plasma loss measured using ^{125}I-labelled sheep albumin. Full details of these calculations have been given earlier.

RESULTS

Bodyweights, Haematological and Biochemical Observations

The average bodyweights of the three groups are depicted in Fig. 1 from which it can be observed that most of the animals continued to grow as the experiment progressed except for the period between the 27th and 31st weeks when the weights of all animals fell due to a reluctance to consume the diet offered during one of the metabolic studies. The performance of sheep exposed to 5,000 cercariae was essentially the same as that of their worm-free counterparts, with both groups exhibiting overall increases in the order of 24-28 kg, but the animals exposed to 10,000 cercariae appeared to experience some retardation of growth, particularly between the 9th and 14th weeks. Although this group subsequently gained weight at a rate similar to the controls, the overall increase was only 20 kg. It is worth emphasising, however, that the performance of these animals during the latter stages is underestimated because of two sheep which became progressively more emaciated during the two months prior to necropsy 35 and 57 weeks after infection; if the changes recorded for these individuals are excluded, the average weight gain of this group was 23 kg.

Venous haematocrit (PCV) was one of the parameters which revealed a clear, if unspectacular distinction between the various groups (Fig. 1), falling from about 35% to 30% in all animals during the first 7 weeks and remaining at around this level in the controls throughout the remainder of the investigation. Infected sheep on the other hand experienced a further drop over the following

4 weeks/

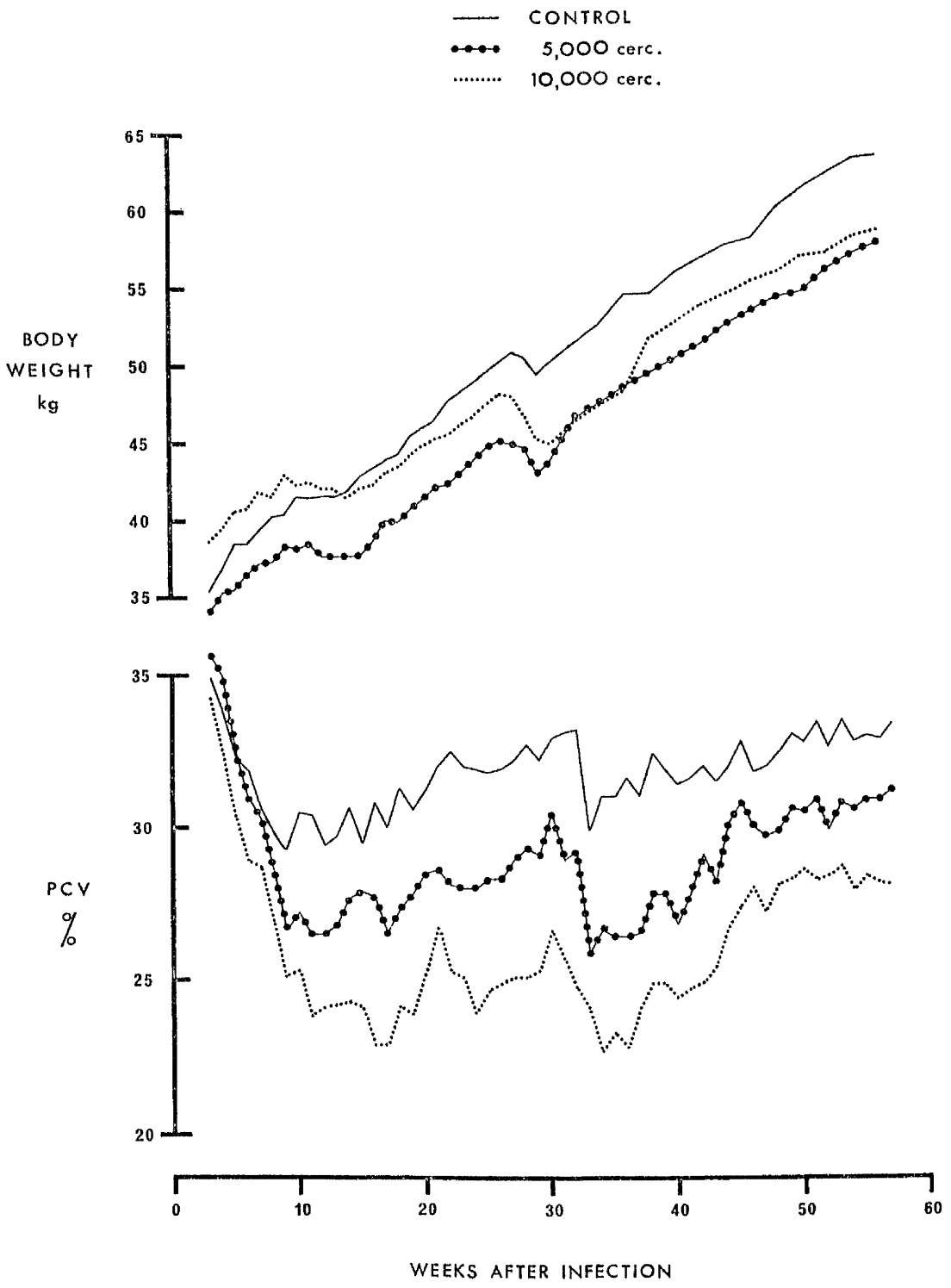

Fig. 1. Body weight and PCV changes in sheep individually infected with either 10,000 or 5,000 S. mattheei cercariae and worm-free controls.

4 weeks, with values reaching about 27% and 24% respectively in sheep exposed to 5,000 and 10,000 cercariae; at this stage both groups were significantly more anaemic than the controls ($p < 0.001$). These values were largely maintained until about the 9th month, but tended to improve subsequently relative to the controls with the result that after 1 year the parasitised animals were essentially haematologically normal.

Serum albumin concentrations were not dramatically influenced by infection although they were generally about 10% lower than normal (Fig. 2). The major difference observed between infected and control sheep with respect to the serum proteins was that the former, and especially those exposed to the higher challenge developed a progressive hyperglobulinaemia between the 2nd and 7th months which persisted over the remaining 5 months of the investigation. Total proteins followed a similar trend with the infected sheep displaying a mild but persistent hyperproteinaemia after the 4th month.

Pathophysiological Data

The results of the blood volume measurements made at various stages during the experiment are illustrated in Fig. 3. These show two features of interest. Firstly, although all three groups experienced an equal drop in circulating red cell volume during the first 12 weeks, only in the infected animals was this accompanied by an increased plasma volume (22% and 16% respectively in the groups exposed to 10,000 and 5,000 cercariae). As a result total blood volumes fell slightly in the controls and increased in the infected sheep, particularly those given the larger dose of cercariae.

Secondly/

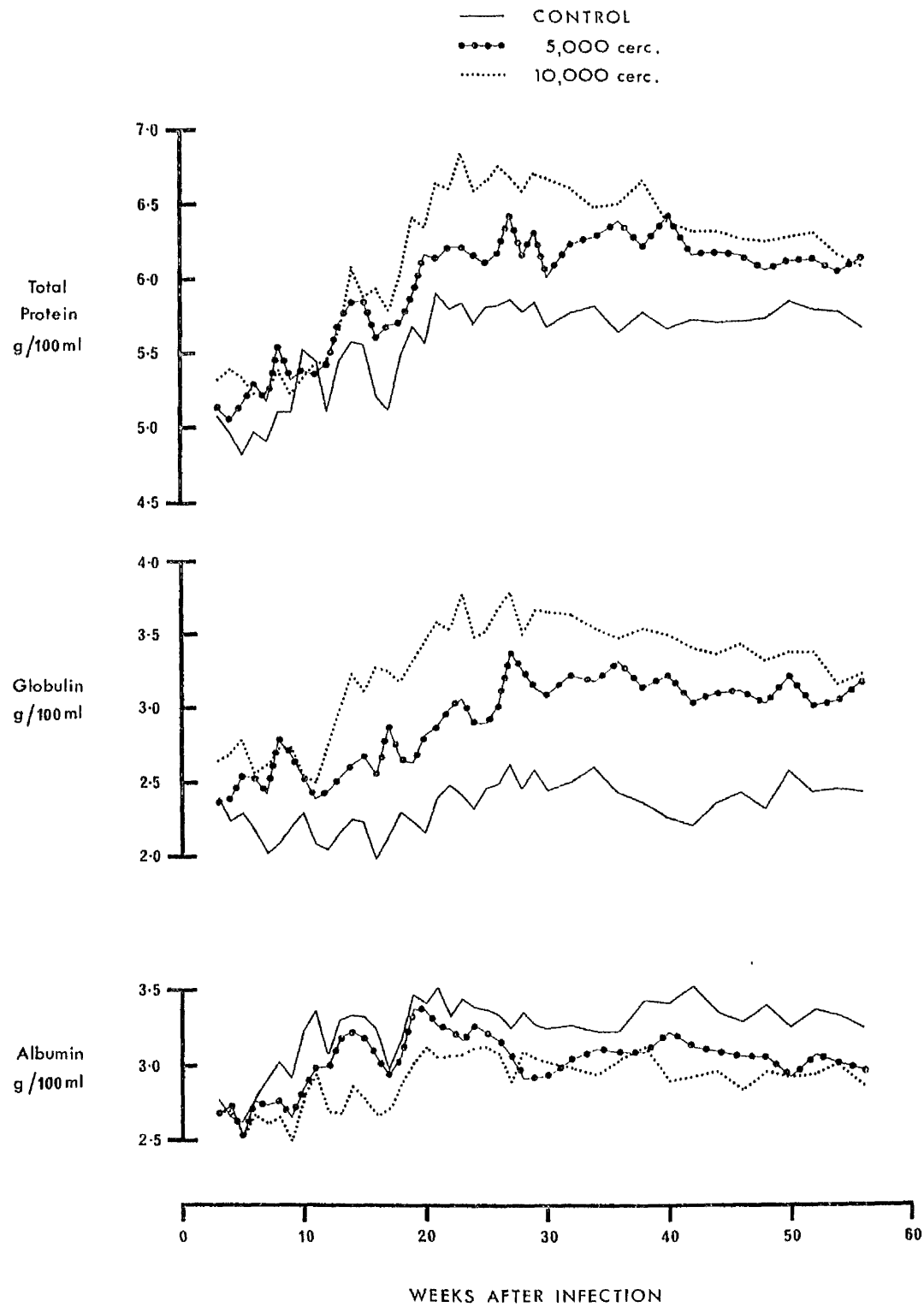

Fig. 2. Serum protein levels in sheep individually infected with either 10,000 or 5,000 S. mattheei cercariae and worm-free controls.

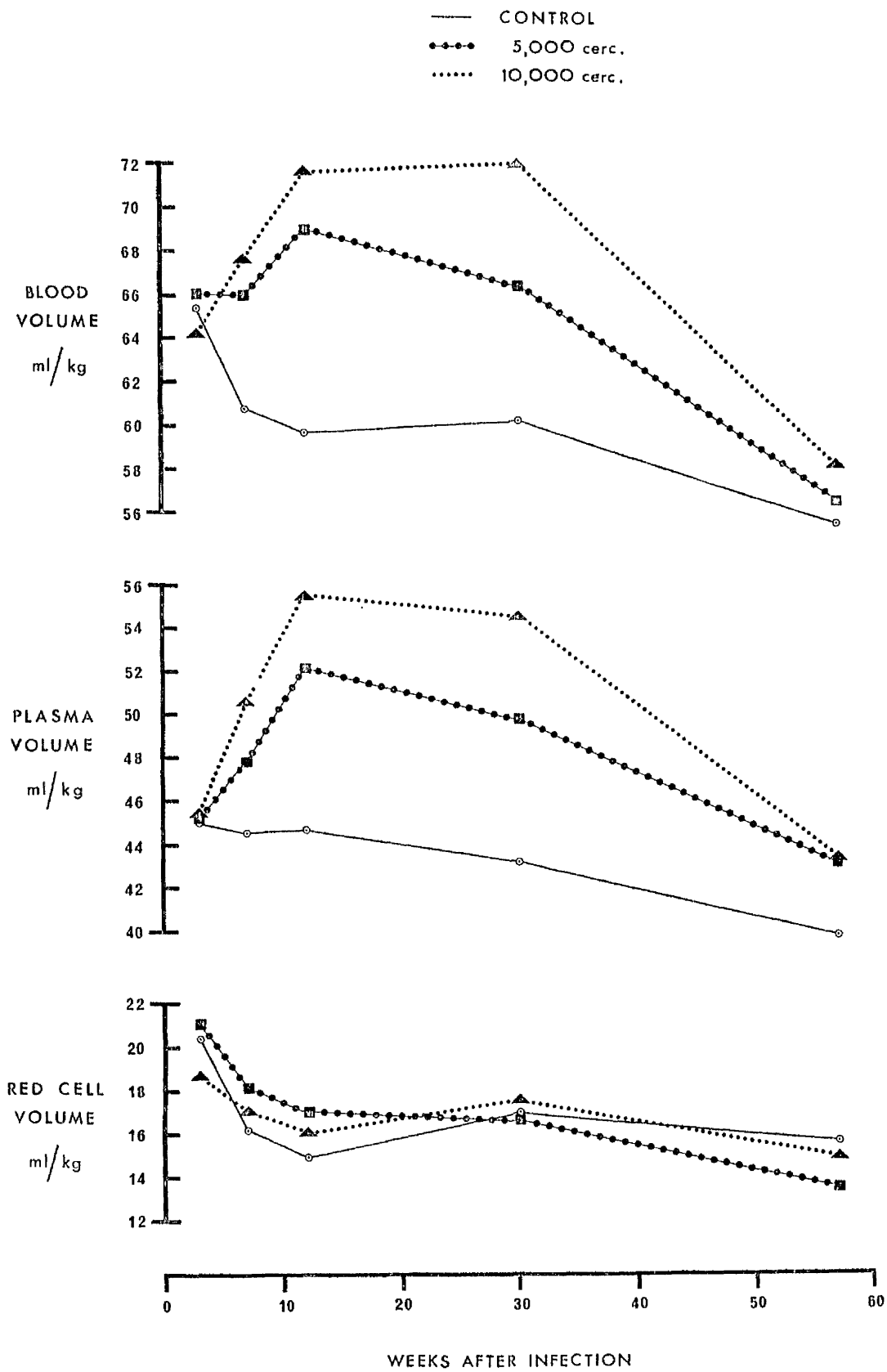

Fig. 3. Blood, plasma, and circulating red cell volumes in sheep individually infected with either 10,000 or 5,000 S. mattheei cercariae and worm-free controls.

Secondly, although plasma and hence blood volumes of both infected groups remained elevated relative to the controls during the following 4 months because of a subsequent sharp drop in plasma volumes of the former, differences between infected and control sheep in the size of plasma and red cell volumes and hence blood volume were minimal by the termination of the study.

The failure of either level of infection to cause significant changes in red cell kinetics is suggested by the data given in Table 1 showing essentially constant rates of red cell synthesis and only small reductions in red cell survival in association with minor haemorrhage into the gut between the 7th and 17th weeks of infection. Despite the presence of mild hypoalbuminaemia, the intravascular pool of albumin in infected sheep remained normal throughout, although there was some evidence of extravascular depletion, especially in the more heavily infected group (Table 2). This probably arose from the slight hypercatabolism noted in these animals between the 2nd and 4th months coincident with an increased protein loss in the gut.

Clinical Observations

Only two infected sheep showed obvious signs of disease, all others remaining bright, in good condition and showing no evidence of inappetence or faecal discoloration. The affected animals, which belonged to the group exposed to 10,000 cercariae, became progressively emaciated, inappetent and anaemic over a period of 4-6 weeks prior to necropsy 35 and 57 weeks after infection, but at no stage were the faeces soft or discoloured.

Table 1: Red Cell Kinetics of Sheep Infected with *S. mattheei* and Controls (Mean ± S.E.)

Weeks After Infection	Group	^{59}Fe Half-life (min)	Plasma Iron Turnover Rate (mg/day/100ml blood)	Apparent Red Cell Half-life (hours)	Faecal "Blood" Clearance (ml/day)
3	10,000	182 ± 13	0.516 ± 0.037	284 ± 20	4.07 ± 1.05
	5,000	158 ± 11	0.447 ± 0.024	342 ± 20	4.45 ± 0.73
	Control	189 ± 16	0.435 ± 0.030	313 ± 14	2.60 ± 0.34
7	10,000	159 ± 15	0.451 ± 0.035	226 ± 11	6.30 ± 1.79
	5,000	142 ± 7	0.491 ± 0.014	214 ± 9	5.32 ± 0.82
	Control	162 ± 10	0.536 ± 0.022	271 ± 19	1.87 ± 0.22
13	10,000	151 ± 14	0.514 ± 0.041	278 ± 43	13.71 ± 5.67
	5,000	114 ± 9	0.605 ± 0.026	292 ± 21	4.39 ± 0.33
	Control	118 ± 4	0.611 ± 0.024	346 ± 15	2.52 ± 0.46
30	10,000	261 ± 49	0.310 ± 0.077	353 ± 62	4.34 ± 0.66
	5,000	176 ± 15	0.400 ± 0.054	340 ± 25	3.40 ± 0.37
	Control	176 ± 22	0.459 ± 0.033	354 ± 17	1.97 ± 0.21
57	10,000	164 ± 14	0.522 ± 0.080	204 ± 17	
	5,000	173 ± 23	0.550 ± 0.061	190 ± 6	
	Control	152 ± 18	0.534 ± 0.022	221 ± 5	

Table 2: Albumin Distribution and Catabolism in Sheep Infected with S. mattheei and Controls (Mean ± S.E.)

Weeks After Infection	Group	CA (g/kg)	EA (g/kg)	Apparent Albumin $t_{\frac{1}{2}}$ (hours)	F(CA)	Faecal "Plasma" Clearance (ml/day)
3	10,000	1.22 ± 0.04	1.79 ± 0.15	426 ± 20	0.090 ± 0.006	19.3 ± 4.2
	5,000	1.20 ± 0.04	2.12 ± 0.12	446 ± 12	0.104 ± 0.012	24.1 ± 6.0
	Control	1.20 ± 0.05	1.77 ± 0.12	419 ± 37	0.087 ± 0.007	22.9 ± 2.9
7	10,000	1.34 ± 0.03	1.42 ± 0.10	346 ± 34	0.075 ± 0.008	22.2 ± 1.6
	5,000	1.31 ± 0.04	1.71 ± 0.08	405 ± 45	0.081 ± 0.007	24.9 ± 3.6
	Control	1.32 ± 0.05	1.85 ± 0.09	478 ± 50	0.065 ± 0.006	15.7 ± 1.1
13	10,000	1.56 ± 0.06	1.75 ± 0.05	434 ± 57	0.059 ± 0.004	26.9 ± 7.8
	5,000	1.52 ± 0.05	1.90 ± 0.09	509 ± 39	0.061 ± 0.004	20.4 ± 1.3
	Control	1.43 ± 0.05	2.00 ± 0.15	610 ± 60	0.055 ± 0.000	11.3 ± 1.1
30	10,000	1.53 ± 0.08	1.58 ± 0.05	651 ± 47	0.062 ± 0.002	12.0 ± 1.6
	5,000	1.48 ± 0.06	1.71 ± 0.07	534 ± 26	0.066 ± 0.002	11.4 ± 1.7
	Control	1.34 ± 0.05	2.19 ± 0.14	789 ± 69	0.083 ± 0.008	8.5 ± 1.4
57	10,000	1.33 ± 0.04	1.95 ± 0.11	451 ± 115		
	5,000	1.28 ± 0.04	1.79 ± 0.12	338 ± 18		
	Control	1.32 ± 0.01	1.82 ± 0.15	396 ± 26		

CA Intravascular Pool EA Extravascular Pool F(CA) Catabolic Rate

Parasitological Data

Eggs appeared in the faeces at 7 weeks after infection in a few individuals given 10,000 cercariae, but never exceeded 5 eggs/g and generally became negative by the 7th month of infection; eggs were not detected in the faeces of any of the sheep given 5,000 cercariae.

The results of the adult worm recoveries and tissue egg counts performed at various stages of the investigation are shown in Tables 3 and 4. These reveal four features of particular interest. Firstly, although almost twice as many worms were recovered at each stage from the animals exposed to the heavier challenge, in both groups the mean parasite burden fell linearly ($p < 0.001$) from 35% of the infecting dose at 13 weeks to 10% on the 57th week. Secondly, despite the reduction in worm load, the proportion of females remained steady at 40-45%. Thirdly, unlike the worm burdens, there was no correlation between tissue egg counts and duration of infection, nor was there any difference between the groups in relative egg distribution, although egg loads of animals infected with 10,000 cercariae were generally about 30% higher (30 million compared with 20 million). Finally, the two sheep which succumbed to infection did not have particularly high worm burdens, but their tissues contained the greatest number of eggs (60-70 million).

Table 3. Necropsy Findings on Sheep Infected with 5,000 S. mattheei Cercariae

Weeks After Infection	Sheep No.	Worm Burden	Worm Pairs	Total Tissue Eggs x 10^6	Distribution of Eggs (%)		
					Liver	Small Intestine	Large Intestine
13	72	1593	736	21.52	11.1	62.6	26.3
	80	1762	853	23.34	10.8	49.4	39.7
	98	1559	730	15.49	16.1	60.2	23.8
	99	1119	504	21.82	7.8	64.4	27.8
	Mean	1508	706	20.54	11.5	59.2	29.4
	S.E.	137	73	1.73	1.7	3.4	3.5
35	50	1167	506	16.68	27.3	56.4	16.3
	82	387	6	0.05	43.0	10.0	47.0
	184	689	130	18.04	13.9	62.9	23.2
	43	845	414	37.20	8.6	54.6	36.9
	Mean	772	264	17.99	23.2	46.0	30.9
	S.E.	162	117	7.60	7.7	12.1	6.9
57	79	356	82	12.43	23.6	56.9	19.5
	185	698	316	20.86	21.7	52.8	25.5
	42	677	336	47.67	5.0	73.4	21.5
	54	569	204	34.10	15.2	69.9	15.0
	89	288	41	9.52	18.6	58.4	23.0
	97	189	24	5.97	27.5	55.8	16.7
	73	647	253	16.56	19.7	62.4	17.9
	59	811	403	45.69	13.5	59.6	27.0
	Mean	529	207	24.10	18.1	61.2	20.8
	S.E.	79	51	5.77	2.4	2.5	1.5

Table 4: Necropsy Findings on Sheep Infected with 10,000 *S. mattheei* Cercariae

Weeks After Infection	Sheep No.	Worm Burden	Worm Pairs	Total Tissue Eggs x 10^6	Distribution of Eggs (%)		
					Liver	Small Intestine	Large Intestine
13	58	3037	1403	40.46	11.6	63.7	24.7
	77	2611	1228	33.00	13.2	46.5	40.3
	Mean	2824	1316	36.73	12.4	55.1	32.5
	S.E.	213	87	3.73	0.8	8.6	7.8
35	* 49	1503	695	69.97	16.5	68.2	15.3
57	2	638	241	5.01	21.2	67.1	11.8
	* 71	482	203	59.61	19.6	56.8	23.6
	94	1291	619	33.34	18.5	77.4	4.2
	Mean	804	354	32.65	19.8	67.1	13.2
	S.E.	248	134	15.76	0.8	5.9	5.6

* Sheep killed *in extremis*.

DISCUSSION

The most striking feature of this study was the almost complete absence of clinical disease in the great majority of infected animals, which, apart from a slight check in growth coincident with the appearance of mild anaemia between the 2nd and 5th months, could not be differentiated from the worm-free controls. This finding is all the more remarkable when viewed against the devastating effects recorded by Preston and co-workers [7] in sheep exposed to the same number of cercariae as some of the animals studied here. Such differences could hardly be explained by a complete failure of infection since the initial worm establishment was high, and in the case of the animals exposed to 10,000 cercariae, considerably greater than the minimum necessary to produce severe clinical illness and early death [3, 13]. Neither, apparently, could they be explained by the presence of sterile infections since large numbers of eggs were deposited in the liver and intestinal tract. Tissue egg densities (eggs/g) in the intestines and liver at 13 weeks were similar to the densities recorded by Preston and co-workers [7] in slightly older infections, and both organs sustained severe damage, at least as judged histologically.

There were, however, two respects in which the present infections differed from those described by previous workers - namely the almost complete absence of eggs in the faeces of sheep used in the current studies, and the marked reduction in worm numbers with increasing length of infection. Both factors obviously played an important part

in/

in ameliorating the clinico-pathophysiological effects of the infections inasmuch as: (a) the few changes recorded, i.e. anaemia, hyperproteinaemia and poor weight gain were generally most apparent between about the 2nd and 5th to 7th months of infection when faecal egg counts and worm burdens were maximal and all animals were experiencing some degree of haemodilution and excessive losses of red cells and plasma proteins in the gut; and (b) the subsequent gradual improvement in the haematological indices, serum proteins, blood volumes and rates of red cell and albumin degradation towards normality in infected animals were all coincidental with the disappearance of eggs from the faeces and loss of a large proportion of their worm populations. The fact that these sheep did not develop acute schistosomiasis, even early in the experiment before the worms started dying off and when large numbers of eggs were present in their tissues supports the previous work of Preston and Dargie [11] showing that haemorrhage caused by egg excretion through the bowel wall was the major aetiological factor in the acute forms of this disease.

A number of factors could explain the discrepancies observed between these and previous studies on S. mattheei in sheep, the most obvious being the different strains of parasite. In this work the cercariae were obtained from snails infected with miracidia obtained from eggs recovered from infected hamsters, whereas in the studies reported by Preston and his colleagues [7] and by Lawrence [13] the source was eggs from infected sheep or cattle. All the indications therefore/

therefore point to the fact that the parasite used here was attenuated by hamster passage with the result that the inherent viability and hence pathogenicity of worm and eggs alike for sheep was reduced. However, in view of the fairly rapid elimination of worms, and the general assumption that such a phenomenum is immunologically mediated it is also possible that being maintained in hamsters over a period of years, this strain lacked the range of antigens necessary to ward off an immunological attack from its new host (see review by Smithers and Terry [19]). A further possibility is that differences in breed susceptibility or resistance were involved, such as occurs with infections with Haemonchus contortus [20]. Whatever the reason, a knowledge of the relative importance of these factors would clearly be useful for a better understanding of this host-parasite relationship. Studies along these lines were therefore conducted under more carefully standardised conditions and the results obtained are presented in the following chapter.

SUMMARY

In a study of the pathogenesis of ovine schistosomiasis, 11 Suffolk X Border Leicester sheep were each infected with 10,000 S. mattheei cercariae, 16 with 5,000 cercariae, and 13 acted as worm-free controls. Blood samples were taken weekly for packed cell volume determination, serum samples were collected weekly during the first 30 weeks and thereafter fortnightly for protein analysis, and the animals were weighed and samples from the rectum obtained at similar intervals for faecal egg counts. At intervals, 4-8 animals from each group were placed in metabolism cages for periods of 2-3 weeks to monitor albumin and red cell turnover. The animals were necropsied at 13, 35 or 57 weeks after infection, their worms recovered by perfusion and various tissues collected for egg counting.

A most unexpected finding was the almost complete absence of clinical signs in most of the infected animals. The only signs of infection being a slight retardation in growth coincident with the appearance of a mild anaemia between the 2nd and 5th months. These findings were quite unlike those reported by previous workers and were remarkable in view of the large cercarial exposure doses used and the heavy worm and tissue egg counts these produced. However, the present infections differed in two respects from those described previously; there was an absence of eggs from the faeces with associated lack of intestinal bleeding, and also large numbers of worms died in the later stages of the infections. These observations imply that the major factor in the aetiology of the severe disease in sheep is intestinal bleeding caused by the passage of eggs through the bowel wall.

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CHAPTER 6

Comparison of the Sequential Development of Disease in Sheep Infected with Different Strains of *S. mattheei*

INTRODUCTION

Preston and his colleagues [1] found that exposure of Romney Marsh sheep to 10,000 cercariae of a strain of *S. mattheei* obtained from Nelspruit, South Africa, resulted in an acute syndrome with fatal consequences. In complete contrast, the results reported in the previous chapter showed that Border Leicester x Suffolk sheep exposed to the same dose of cercariae of the Nelspruit parasite, remained comparatively healthy. It was thought that this might be due to the different breeds of sheep used in the two studies or that the Nelspruit parasite had become attenuated. The current investigation was planned to determine whether hamster passage of the parasite or the breed of sheep was responsible for altering the parasites' pathogenicity. The hamster maintained strain was therefore compared in Romney Marsh sheep with a recently imported strain maintained exclusively in sheep at Onderstepoort in South Africa.

MATERIALS AND METHODS

Strains of S. mattheei

Two strains of S. mattheei were used, designated MT and VW. The MT strain was obtained from Dr. J. Pitchford in Nelspruit in 1973 and subsequently maintained at Winches Farm, St. Albans, England in hamsters and Bulinus globosus snails from Nelspruit. It was originally isolated from naturally infected snails at Komatipoort in 1957 and subsequently maintained for 2 years in mice and then in Mastanys natalensis, (Pitchford, personal communication); cercariae shed by snails infected with this strain in Nelspruit in 1972 were used to infect the sheep studied by Preston and his colleagues[1]. The VW strain was obtained from Bulinus globosus snails infected by Dr. J. van Wyk at Onderstepoort with miracidia from sheep; this strain was brought to Onderstepoort from Zululand in 1963 and passaged subsequently in sheep, (van Wyk, personal communication).

Experimental Animals and Design

Thirteen six-month old, parasite-free, Romney Marsh sheep were used in the study being fed hay supplemented with concentrates and allowed free access to water throughout. Five sheep were exposed percutaneously to 10,000 cercariae of the VW strain, and a further five animals similarly infected with the MT strain; the remaining three animals acted as controls. The sheep were weighed and bled weekly for serum collection and PCV determinations; and faecal samples

from/

from the rectum were examined for schistosome eggs as described previously. At 10, 41, 60 and 83 days post-infection measurements of plasma and circulating red cell volumes and plasma iron turnover rates were made using ^{125}I-labelled albumin, ^{51}Cr-labelled red cells and ^{59}Fe as described in earlier chapters.

One of the VW sheep became sick and was perfused at 62 days together with one MT sheep (for comparative purposes); the remainder were perfused 13 $13\frac{1}{2}$ weeks after infection. The techniques used for perfusion and tissue egg counts were similar to those described previously.

RESULTS

The results presented in the figures are the average values of the three groups of sheep; individual data are given in Appendix 6.

Clinical Observations

When examined terminally the sheep infected with the VW strain appeared distinctly dull, their wool was poor and in some cases broken. Tachycardia and hyperpnoea were constant features and some animals experienced spasms of unproductive coughing. Faecal pellets were soft and coated with strands of blood-stained mucus which tended to bind the pellets together. Few of these features were observed in the MT and control groups which were bright and in good condition and passed firm pelleted faeces with only occasional evidence of discolouration due to haemorrhagic material in the former.

Faecal Egg Counts

Eggs first appeared in the faeces of the VW sheep 7 weeks after infection, all were positive by the 8th week, and by the 12th week the mean egg count of this group was 300/g (Fig. 1). By contrast only two of the sheep infected with the MT strain became positive (this was delayed until week 9) and in both the maximum count was only 50/g.

Body Weights and Packed Cell Volumes

Bodyweights (Fig. 2) deviated little from control values

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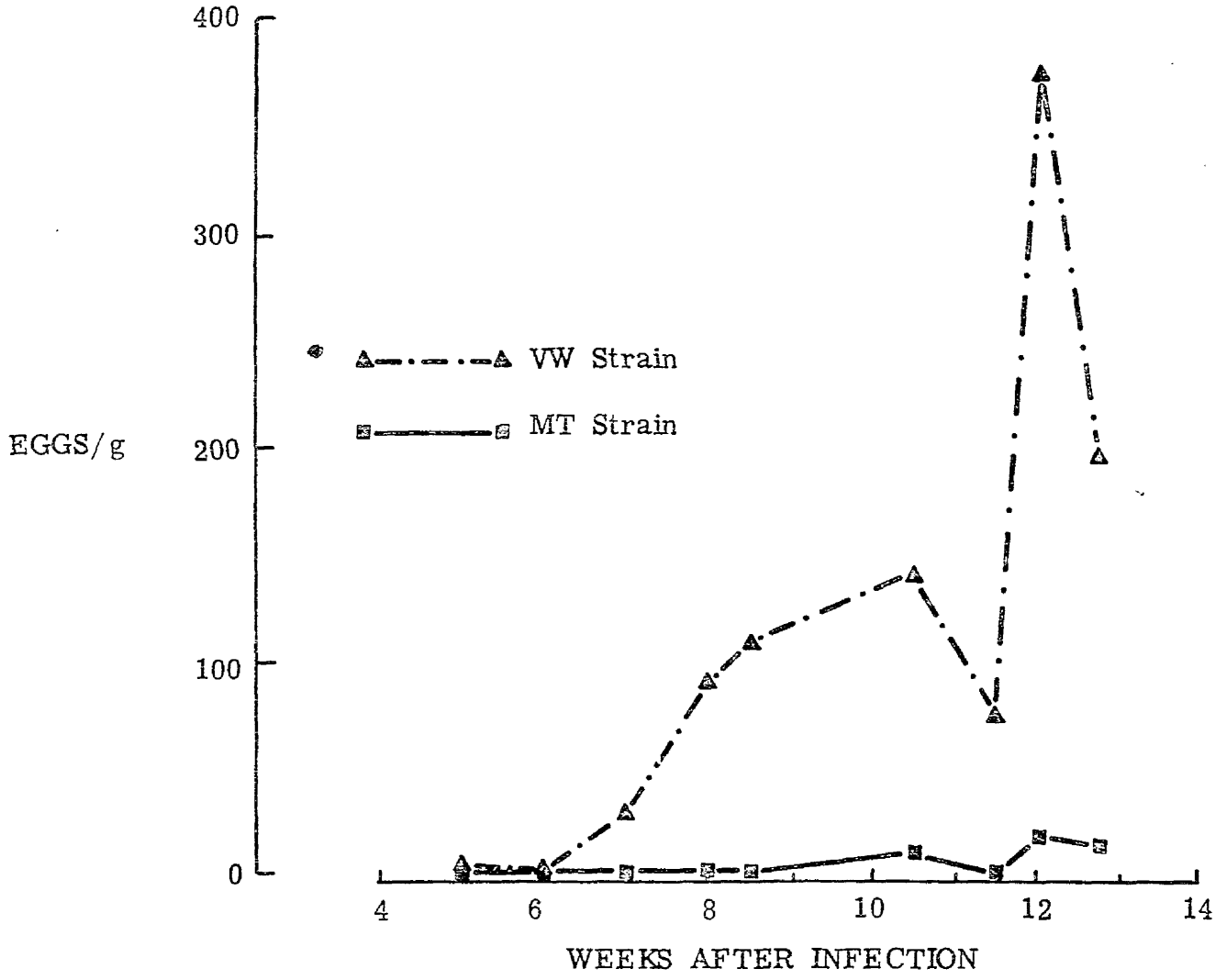

Fig. 1 : Eggs Recovered from the Faeces of Sheep Infected with Two Different Strains of S.mattheei.

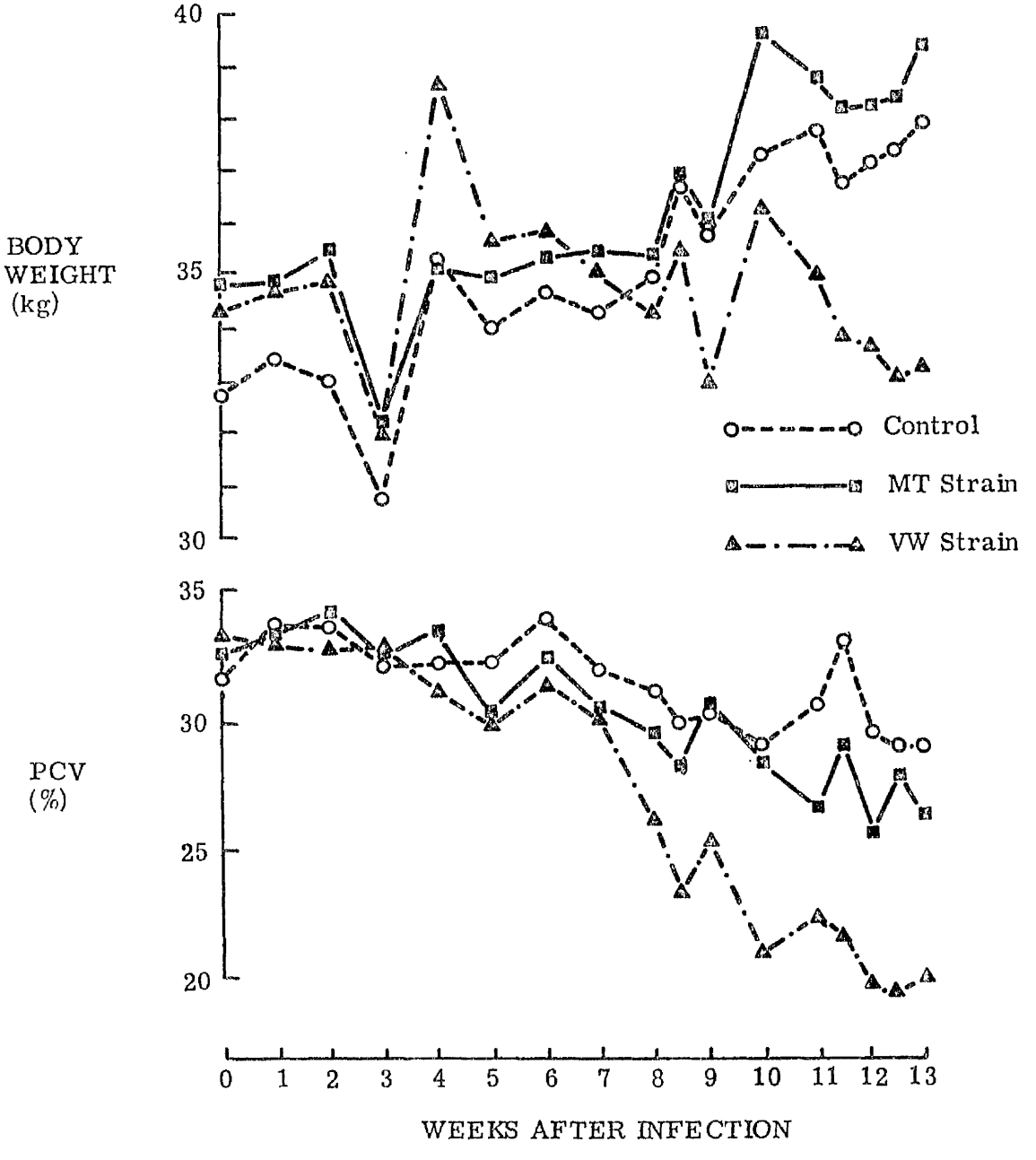

Fig. 2 : Body Weight and PCV Changes in Sheep infected with Two Different Strains of S. mattheei.

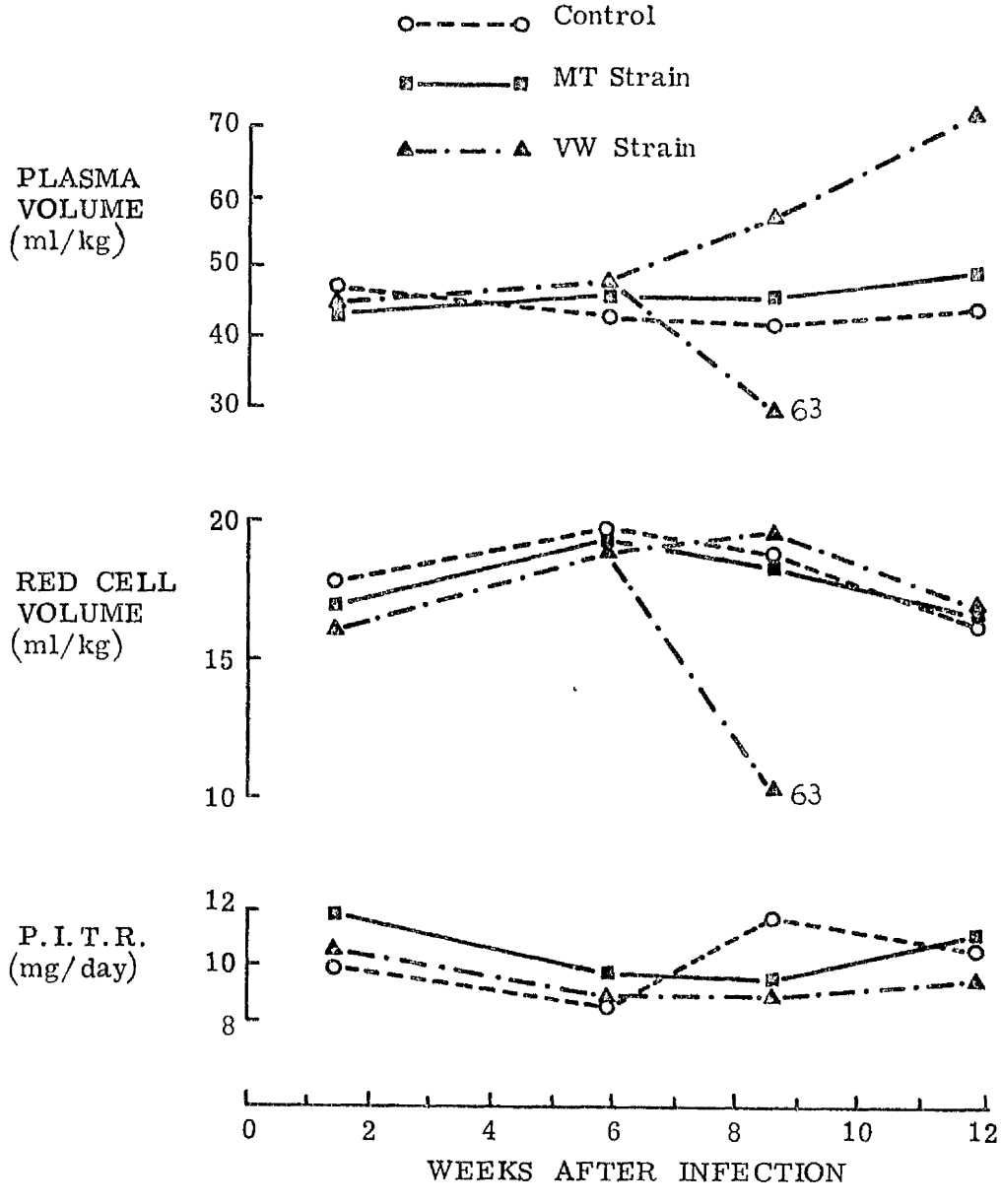

Fig. 3 : Plasma and Red Cell Volumes and Plasma Iron Turnover Rates in Sheep Infected with Two Different Strains of S. mattheei.

during the first 8 weeks of the investigation, but subsequently fell in the VW group and increased in the MT and control groups. By the 12th week, VW sheep were significantly ($P < 0.05$) lighter (about 5 kg or 15%) than both the MT and control sheep.

PCV values (Fig. 2) of all three groups were well maintained at about 32% over the initial 7 weeks of infection, falling dramatically thereafter in animals infected with the VW strain (to 20% on week 12). Sheep 63 which wasnecropsied about the 9th week had a terminal PCV of 13%. Some deterioration was also noted in the MT group but this was slight (PCV's of 27% compared with 30% in the controls on week 12).

Blood Volumes and Plasma Iron Turnover Rates

Plasma volumes (Fig. 3) increased sharply by almost 50% in 4 of the VW sheep between the 6th and 12th weeks, but No. 63 which developed an acute bloody diarrhoea exhibited terminal hypovolaemia. Plasma volume changes in the MT and control groups were minimal.

No marked changes in either the circulating red cell mass or plasma iron turnover rate (Fig. 3) were recorded in any of the sheep, with the exception of No. 63 which experienced a dramatic reduction in red cell mass prior to necropsy.

Worm Recovery and Tissue Egg Counts

At perfusion, worm recovery was 47% in the VW strain sheep and only 24% in the MT strain (Table 1). In addition almost

twice/

Table 1: Worm and Tissue Egg Counts in Sheep Infected with Different Strains of *S. mattheei*

Group	Sheep No.	Individual Worm Burdens	Individual Total Tissue Egg Counts ($\times 10^6$)	Total Tissue Eggs/Worm Pair ($\times 10^3$)	Distribution of Tissue Eggs (%)			
					Liver	Small Intestine	Large Intestine	Lungs
VW Strain	63+	3832	13.6	11.0	12.7	65.5	21.8	N.D.
	64	4241	31.5	22.6	8.8	72.0	19.2	0.0
	65	5334	16.0	9.5	9.1	76.1	14.8	0.1
	66	5096	31.5	18.6	3.7	71.0	25.3	0.0
	67	4859	33.2	17.6	1.8	85.2	12.9	0.1
	Mean	4672	25.2	15.9	7.2	74.0	18.8	0.1
	S.E.	278	4.3	2.4	2.0	3.3	2.3	0.0
MT Strain	68	2574	9.3	7.8	11.0	62.0	27.0	0.0
	69	2065	14.0	15.0	13.0	57.8	29.2	0.0
	70+	3171	6.6	5.8	29.7	44.2	26.1	N.D.
	71	2116	16.1	16.7	21.6	58.0	20.4	0.0
	72	1936	22.9	25.1	13.8	48.8	37.3	0.0
	Mean	2372	13.8	14.1	17.8	54.2	28.0	0.0
	S.E.	227	2.8	3.4	3.5	3.3	2.7	0.0

\+ Sheep killed at 62 days after infection

N.D. Not determined

twice as many eggs were present in the tissues of the VW sheep and although there was little difference in "eggs/worm pair" calculated from the tissue egg counts, the overall fecundity of the VW worms was much higher than the MT strain because of the large numbers of eggs excreted in the faeces by the VW sheep (each of the VW sheep excreted about four million eggs during patency whereas with the MT strain the two positive animals passed no more than 0.1 million eggs each).

There were also marked differences in the relative distribution of eggs in the tissues, many more eggs of the MT strain being found in the liver and although some eggs were generally present in the lungs of the VW sheep none were found in the MT strain sheep.

DISCUSSION

Sheep infected with S. mattheei which had been maintained exclusively in sheep showed similar signs of disease to those studied by Preston and his colleagues [1-3], with sharp decreases in weight and PCV and increases in plasma volumes. No comparable changes occurred in the sheep exposed to the parasite which had been maintained in hamsters and apart from sporadic eggs in the faeces the only sign of infection was a slight drop in PCV towards the end of the experiment. Since the behaviour of this latter strain was similar to that recorded in the previous chapter with a different breed of sheep, it seems likely that its loss of virulence was attributable to its maintenance in hamsters.

Although the results of the worm and egg counts show that the difference in virulence can partly be accounted for by the higher infectivity and fecundity of the sheep strain, it seems likely that the major factor contributing to the low pathogenicity of the hamster strain in sheep was the small number of eggs able to pass through the intestinal mucosa into the lumen of the gut. In the previous chapter, the almost total absence of eggs in the faeces of sheep infected with the hamster strain was also associated with very little intestinal haemorrhage. It is not clear why the eggs of the hamster strain of S. mattheei failed to reach the gut lumen, but presumably the eggs were either not deposited appropriately by the female worms because of a behavioural change, or the miracidia within the eggs are lacking in some of the enzymes which normally facilitate

egg/

egg excretion. There were also differences in egg deposition in other tissues, 15% of the total eggs of the hamster strain being deposited in the liver compared with only 6% in the sheep strain. The fact that much more difficulty was experienced in perfusing sheep strain worms from the bowel wall cappillaries than with the hamster strain, suggests that the sheep strain worms penetrate more deeply into the bowel wall.

Infraspecific strain differences are thought to be of fundamental importance in the epidemiology of many helminth infections [4] and while strain differences have been documented for S. japonicum, S. mansoni and S. haematobium this is the first recorded for a schistosome species of veterinary importance. Strain differences described for the human parasites include profound differences in infectivity to different strains of snail and definitive hosts as well as clear differences in pathogenicity. The most spectacular example of the latter is the total failure of Taiwanese S. japonicum to mature in man, whereas other strains of this parasite are of course highly pathogenic [5]. Other studies include those of Nelson and Saoud [6] on S. mansoni in rhesus monkeys, Wright and Bennett [7] on S. haematobium in hamsters, and in most of these experiments laboratory rodent-passaged schistosomes were used. It is clear from the present study that hamster passage can quickly modify the parasites dramatically, so it would be unwise to assume that laboratory strains behave in the same way as the parasites in nature. Saoud [8], for example, found that an Egyptian strain of S. mansoni maintained in laboratory animals for over twenty years was markedly different from

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a recently isolated Egyptian strain; it had lost its infectivity to its original snail host but in this case it was the old laboratory strain which was more pathogenic to mice. Saoud [9] attributed the changes in his parasites to adaptation to an abnormal snail host (Biomphalaria glabrata instead of B. alexandrina) but maintenance in an abnormal snail host cannot explain the change of S. mattheei documented in the present study as the parasite was throughout maintained only in the original snail host, Bulinus globosus from Nelspruit.

SUMMARY

The present study was carried out to determine whether the absence of clinical symptoms in Border Leicester x Suffolk sheep following their infection with a strain of S. mattheei previously found to be pathogenic in Romney Marsh sheep was due to differences in the breeds of sheep or the maintenance of the parasite in hamsters. Five Romney Marsh sheep were each infected with 10,000 cercariae of the hamster-passaged parasite and five with 10,000 cercariae of a S. mattheei strain from Onderstepoort, South Africa, which had been passaged exclusively through sheep.

Striking differences in pathogenicity were found between the two strains. Infection with the "sheep" strain was lethal, whereas infection with the "hamster" strain produced little evidence of clinical disease. Considerable parasitological differences were noted between the two strains: egg production began at 7 weeks with the sheep strain, faecal counts rising to more than 300 eggs/g, while only two of the sheep infected with the hamster strain produced eggs in the faeces (at 9 weeks) and the maximum egg count was 50 eggs/g. Twice as many adult worms of the sheep strain were recovered, and, although the number of eggs found in the tissues "per worm pair" was not significantly different, overall egg production was higher for the sheep strain, due to the markedly higher faecal egg output. The percentage distribution of eggs in the tissues was also different, more of the sheep strain eggs being deposited in the intestines than for the hamster strain.

The/

The results suggest that the parasite had become attenuated, presumably, due to its maintenance in hamsters. The faecal egg counts indicated an inability of hamster strain eggs to penetrate the intestinal lumen, possibly due to a behavioural mutation of the worms or enzyme deficiencies in their eggs. This failure of egg excretion was the important factor in reducing the pathogenicity of the hamster strain.

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CHAPTER 7

Immunization of Sheep Against a Virulent Strain of S. mattheei, Using a strain of S. mattheei Attenuated by Hamster Passage

INTRODUCTION

The possibility of vaccinating domestic animals against schistosomiasis is currently being investigated since it is now recognised that the disease can be of considerable veterinary importance, and present methods of treatment and control are unsatisfactory. The first observation suggesting that domestic livestock could develop resistance to this disease was made by Fujinami[1] who noted that cattle and horses which recovered from natural or experimental infections of S. japonicum were resistant to subsequent reinfection. This stimulated considerable interest in the immunology of schistosomiasis most of which centred on the parasites of particular importance to man. A number of reviews on the subject have appeared recently [2, 3, 4], from which it is clear that although many experimental host species can develop some degree of resistance against reinfection, both the level and speed of acquisition of immunity varies from one host-parasite system to another. The situation as regards domestic animals is even more obscure, largely because of an almost total lack of sound epidemiological investigation and the relatively late recognition of the veterinary and zoonotic importance of the disease in these species.

So far, three basic protocols have been followed in attempting to stimulate immunity in calves and sheep against their own schistosomes. The simplest consists of infection followed by reinfection with homologous cercariae. The results obtained could at best be described as moderately encouraging

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in that although there was often little or no reduction in worm establishment from the challenge dose the capacity of these worms to produce eggs was invariably reduced to the extent of preventing clinical disease [5, 6].

The second technique is to stimulate resistance by prior exposure to non-pathogenic schistosome species. This possibility first arose from epidemiological observations suggesting the existence of cross immunity between different species of schistosomes, e.g. between S. bovis and the parasites of man, S. haematobium and S. mansoni [7, 8]. More recently, the phenomenon (now known as "heterologous" immunity) has been confirmed experimentally with the demonstration that repeated exposure of monkeys to S. bovis induced a high level of protection (80%) against subsequent infection with S. haematobium [9]. However attempts to vaccinate calves and sheep against S. mattheei and S. bovis by a single exposure to S. mansoni have met with varying degrees of success [10, 11, 12].

Nevertheless, results from such studies did indicate that the presence of adult egg-producing worms was not a pre-requisite for the development of acquired immunity to either parasite and raised the possibility of developing a vaccine incorporating cercariae or schistosomes attenuated by irradiation, thereby precluding the attainment of sexual maturity i.e. a procedure essentially similar to that first developed for the successful prevention of parasitic bronchitis in cattle [13] and sheep [14] and hookworm in dogs [15]. So far however, the results obtained/

obtained have been somewhat less encouraging although, to be realistic, the technique is still in the embryonic stage of development and fraught with technical difficulties.

The original work along these lines was carried out by Taylor and his colleagues [12] using sheep exposed to four doses each of 10^4 irradiated S. mattheei cercariae or schistosomules, and challenged with 5,000 normal cercariae. Worm burdens of the vaccinates were reduced by about 75% and tissue egg counts by 50% compared with challenge controls, but no significant differences were seen in liver pathology. When the procedure was repeated using only two doses of irradiated cercariae the degree of protection was reduced to 10%. Unfortunately none of their animals was examined clinically and both the vaccinating and challenge infections were derived from the same hamster strain as used in the studies reported in an earlier chapter of this thesis. Bearing in mind the results of these investigations, it is clear that confirmation of the efficacy of this vaccine against a pathogenic strain of the parasite is required.

From the information given above it is apparent that although active immunisation against schistosomiasis may yet be possible, the efficacy of any procedure must be quantified more vigorously than hitherto. So far, the approach has been almost entirely one-sided, being orientated toward "protection against schistosomes" with little regard as to whether the procedure also "protects against schistosomiasis". As the results of previous chapters demonstrate, the two are not synonymous. Sheep can in fact remain clinically healthy even when heavily infected with schistosome worms and eggs, provided of course that/

that these eggs do not appear in the faeces. This observation raises the question of whether the presence of such infections could confer any resistance against homologous challenge with cercariae known to be pathogenic. Whilst such a protocol is unlikely to have any practical value, its potential as a model for future studies at least seemed worthy of investigation. In this chapter, the response of sheep infected with the non-pathogenic strain of S. mattheei to reinfection with the homologous pathogenic sheep strain was therefore assessed by parasitological, clinical and radioisotopic methods, and compared with that of worm-free animals exposed only to the latter strain.

MATERIALS AND METHODS

Experimental Animals and Design

Twelve 1 year old Suffolk cross wethers were drenched with Thiabendazole (Thibenzole, Merck, Sharp and Dohme Ltd., Hoddesdon, Hertfordshire, England) and divided into three equal groups. Members of one group were vaccinated with 10,000 cercariae of a strain of S. mattheei maintained in hamsters, and along with a group of worm-free sheep challenged 63 weeks later with 10,000 S. mattheei cercariae obtained from snails infected with miracidia from sheep: the third group acted as worm-free controls and all the sheep were necropsied after 16 weeks.

Following infection with the sheep strain the animals were weighed and bled weekly for PCV and serum protein estimations, and egg counts were carried out regularly on samples of faeces taken from the rectum. Red cell and albumin turnover were monitored for 2 weeks immediately before challenge and for a similar period prior to necropsy when the adult worms were recovered by perfusion and tissues collected for pathological examination and egg counts. All the techniques used are described in previous sections.

Apart from the periods when the turnover studies were conducted and the sheep were confined to metabolism cages, the animals were maintained in pens with slatted floors; the diet consisted of hay plus supplementary concentrates and water was available ad lib.

RESULTS

Body Weights, Haematological and Biochemical Observations

During the first 5 weeks, bodyweights of all animals increased at a similar rate, but whereas the controls and to slightly lesser extent the vaccinated sheep continued to grow or at least maintain their weight as the experiment progressed, the unvaccinated animals gradually lost weight (Fig. 1). By the end of the investigation the controls had gained on average 5 kg and the vaccinates 3 kg, while the unvaccinated sheep had lost 7 kg. These changes were accompanied by equally dramatic alterations in PCV, which fell during the study by 19% in the vaccinates and by 44% in the unvaccinated group (Fig. 1). In all infected animals PCV's deteriorated sharply at first and then more slowly, but a notable feature was that the initial drop occurred somewhat earlier in the unvaccinated group i.e. between the 7th and 12th weeks compared with between the 9th and 13th weeks in the vaccinates.

The serum protein levels recorded during the study are also of some interest (Fig. 2). The albumin concentrations of the unvaccinated sheep remained steady until the 6th week but during the following 4 weeks declined sharply by 44% and remained at this new level until necropsy. The vaccinated animals also became hypoalbuminaemic and although the level of this protein had exhibited a tendency to decline between the 2nd and 8th weeks the major reduction, in common with the anaemia, occurred later, i.e. after the 8th week and was less severe than that recorded in the unvaccinated sheep, levels being reduced

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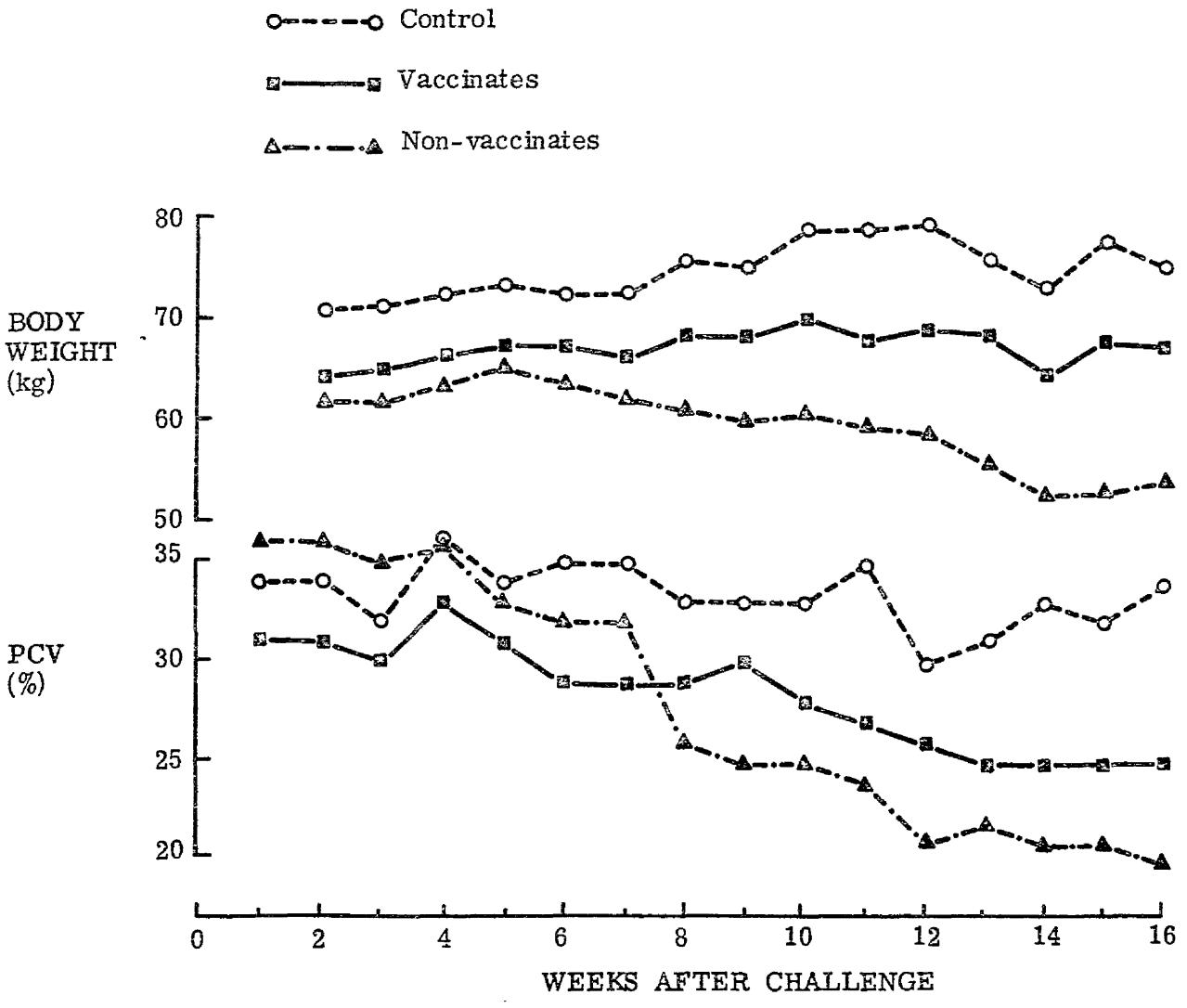

Fig. 1 : Body Weight and PCV Changes in Sheep Following Challenge with a Virulent Strain of S. mattheei.

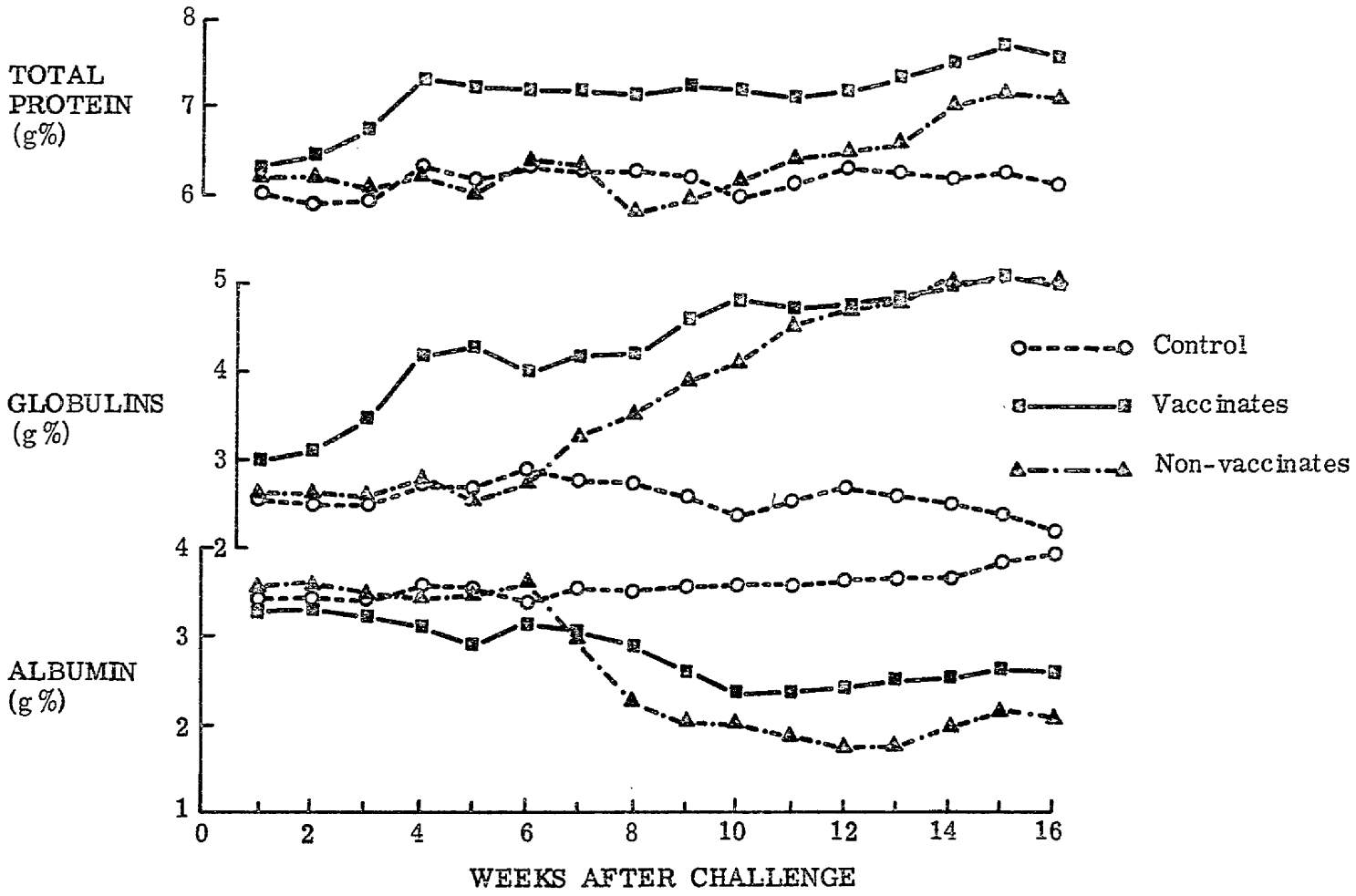

Fig. 2 : Serum Protein Changes in Sheep Following Challenge with a Virulent Strain of S. mattheei.

by only 21% at necropsy. Both groups of infected sheep also exhibited hyperglobulinaemia, but again the timing and rate of development of this feature differed. In the vaccinated animals the major increase in globulin levels occurred between the 2nd and 10th weeks, whereas in the unvaccinated group this was noted between the 6th and 14th weeks. Despite the latter's slower response, globulin concentrations were almost identical in both groups at necropsy; since the vaccinated sheep were already relatively hyperglobulinaemic before challenge, the increase displayed by the unvaccinated animals was actually much more pronounced (i.e. 89% compared with 66%). Total proteins followed a trend similar to that of the globulins with hyperproteinaemia being evident in the vaccinates from the 3rd week onwards while this feature was only evident in the unvaccinated sheep during the later stages of the study.

Pathophysiological Data

The results of the blood volume measurements made immediately before challenge and repeated 2 weeks before necropsy using ^{125}I-albumin and ^{51}Cr-red cells are shown in Table 1. These demonstrate three features of interest. Firstly, that before challenge, the plasma and circulating red cell volumes of the vaccinated sheep were essentially normal. Secondly, although these animals experienced some expansion in plasma volume and reduction in red cell mass following reinfection (25% and 10% respectively) neither of these changes was as severe as recorded in the unvaccinated group (57% and 24% respectively)./

Table 1: Blood Volume Changes Before and After Challenge

Group	Sheep No.	Pre-challenge			16 Weeks Post-challenge		
		Vp ml/kg	RCV ml/kg	BV ml/kg	Vp ml/kg	RCV ml/kg	BV ml/kg
Controls	55	36.7	14.1	50.8	33.1	12.9	46.0
	51	32.8	13.6	46.4	35.2	14.2	49.4
	32	38.3	15.4	53.7	32.9	14.9	47.8
	8	37.4	17.8	55.2	34.3	19.4	53.7
	Mean	36.3	15.2	51.5	33.9	15.4	49.2
	S.E.	1.2	0.9	1.9	0.5	1.4	1.6
Vaccinates	7	41.2	17.6	58.8	43.8	16.7	60.5
	52	44.7	17.3	62.0	55.2	14.4	69.6
	60	40.0	15.0	55.0	55.3	14.0	69.3
	19	34.6	16.3	50.9	46.2	14.5	60.7
	Mean	40.1	16.6	56.7	50.1	14.9	65.0
	S.E.	2.1	0.6	2.4	3.0	0.6	2.6
Non-Vaccinates	11	37.8	17.4	55.2	65.6	11.0	76.6
	95	35.3	18.6	53.9	53.0	16.2	69.2
	68	38.7	17.1	55.8	60.7	12.1	72.8
	190	40.9	15.9	56.8	59.9	13.6	73.5
	Mean	38.2	17.3	55.4	59.8	13.2	73.0
	S.E.	1.2	0.5	0.6	2.6	1.1	1.5

respectively). Thirdly because of such disproportionate increases in plasma volume, all infected sheep, but especially the unvaccinated animals were hypervolaemic relative to the controls and as a result exhibited a much more severe drop in PCV and albumin concentration than in total circulating red cell and albumin mass. In fact although the intravascular pool of albumin (denoted as CA in Table 2) was 4% and 25% smaller relative to the controls in the vaccinated and unvaccinated groups respectively; when related to bodyweight its size was comparable in all 3 groups. In other words, the anaemia and hypoalbuminaemia which developed after challenge were due largely to the dilution arising from excessive fluid retention within the bloodstream. However, that this was not the only factor involved is indicated by the additional data given in Table 2. From these it is clear that following challenge all infected animals but again especially those which were not vaccinated experienced excessive red cell breakdown and catabolism of albumin as assessed by the survival or half-life ($t\frac{1}{2}$) of ^{51}Cr-red cells and ^{125}I-albumin in their circulation; furthermore, as shown by the faecal "clearance" figures, both features were clearly associated with an increased loss of these constituents into the gut.

Clinical Observations

At the end of the experiment the sheep were examined clinically without knowledge of the groupings. The controls were bright and in good condition and with one exception had a body condition score of 4. (Body condition scoring, as defined by/

Table 2: Pathophysiological Parameters Before and After Challenge

Group	Sheep No.	Pre-Challenge CA (g)	Pre-Challenge CA (g/kg)	Pre-Challenge Albumin $t_{\frac{1}{2}}$ (days)	Pre-Challenge Red Cell $t_{\frac{1}{2}}$ (days)	Post-Challenge CA (g)	Post-Challenge CA (g/kg)	Post-Challenge Albumin $t_{\frac{1}{2}}$ (days)	Post-Challenge Plasma Clearance (ml/day)	Post-Challenge Red Cell $t_{\frac{1}{2}}$ (days)	Post-Challenge Red Cell Clearance (ml/day)
Control	55	94.1	1.31	17.3	9.2	103.9	1.32	16.5	22.3	9.5	2.7
	51	65.5	1.04	14.2	8.5	68.5	1.05	14.2	11.3	12.2	2.0
	32	94.7	1.28	15.7	11.3	100.7	1.26	17.6	33.7	13.3	2.6
	8	102.0	1.32	18.1	9.6	99.5	1.26	17.8	26.8	7.6	2.8
	Mean	89.1	1.24	16.3	9.7	93.2	1.22	16.5	23.5	10.7	2.5
	S.E.	8.1	0.07	0.9	0.6	8.3	0.06	0.8	4.7	1.3	0.2
Vaccinates	7	110.4	1.43	18.6	10.4	94.7	1.24	13.0	59.3	7.3	4.6
	52	106.0	1.58	14.3	8.5	107.8	1.49	12.3	71.3	7.2	4.1
	60	80.0	1.36	15.3	7.4	70.6	1.17	13.1	36.7	4.3	8.3
	19	60.6	1.03	14.2	10.9	84.3	1.34	15.5	47.5	9.8	3.6
	Mean	89.3	1.35	15.6	9.3	89.4	1.31	13.5	53.7	7.2	5.2
	S.E.	11.7	0.12	1.0	0.8	7.9	0.07	0.7	7.5	1.1	1.1
Non-Vaccinates	11	80.7	1.35	15.0	8.3	55.0	1.12	10.6	58.3	6.1	9.8
	95	88.2	1.32	18.6	7.8	73.1	1.26	16.0	69.3	5.9	5.7
	68	86.4	1.42	15.9	9.8	58.5	1.03	12.4	48.3	6.5	5.7
	190	81.8	1.39	16.5	9.2	95.0	1.77	10.8	95.5	5.0	12.9
	Mean	84.3	1.37	16.5	8.8	70.4	1.30	12.5	67.9	5.9	8.5
	S.E.	1.8	0.02	0.8	0.4	9.1	0.17	1.3	10.2	0.3	1.7

by the Meat and Livestock Commission.) In general the wool was good with little harshness and reasonable springiness (these terms are used in accordance with the wool grade specifications defined by the British Wool Marketing Board). In all cases the heart rate was 85 - 90/minute with a good volume and regular pulse. Mucous membranes were pink, respiratory rates were normal (40/minute) and there was no coughing. The faeces were small, firm and pelletted, and there was no evidence of discoloration due to haemorrhagic material.

Vaccinated animals were also bright and in good condition with body condition scores ranging from 3 to 3.5. In general the condition of the wool was only moderate and whilst staple length was acceptable, some harshness and loss of springiness was apparent; some dry white areas were observed but the wool was firmly attached. The heart rate of this group ranged from 90 - 110/minute, pulse volume was good in every case and mucous membranes were pink. Respiratory rates at rest were normal except for one animal which had a rate of 70/minute; this animal coughed occasionally and on auscultation rhônchi and occasional râles were heard low on both sides of the chest. The faeces of this group were generally small, firm and separate, but on close inspection they had a very fine coating of muco-haemorrhagic material giving them a shiny, dark-red appearance.

All unvaccinated animals were thin with a body condition score ranging between 2 and 2.5. In general their wool was poor with a reduced length of staple, increased harshness and loss of springiness and whilst some animals showed frank loss of wool, particularly/

particularly over the shoulder and chest, others had areas of dry white wool which was readily pulled out. The demeanour of the animals was variable with two appearing dull and showing little interest in their surroundings, and the others bright. As a result of loss of body weight the eyes were sunken and all animals showed excess lacrymation. In every case there was tachycardia, resting heart rates ranging from 130 - 140/minute, but with one exception which had a chronic pneumonia, there was no evidence of exercise intolerance. Although fast, the heart rate was regular in all animals, but pulse volume was reduced in some instances. Pallor of visible mucous membranes was apparent in only one animal. Respiratory rates at rest were normal but hyperpnoea was obvious. This is a feature commonly observed in anaemic animals which although having a tachycardia do not become tachypnoeic until terminally when they are also dyspnoeic. The animal in the poorest condition (No. 190) was pyrexic (104.6^{o}F), was subject to frequent spasms of unproductive coughing and on auscultation widespread sibilant rhônchi were present. The major clinical feature apparent in this group concerned their faeces. Abnormalities were observed in the size and consistency of faecal pellets which were large and coated with variable amounts of muco-haemorrhagic material which tended to bind the pellets together.

Parasitological Data

Egg counts on the faeces were negative in the vaccinated sheep until 7½ weeks after challenge when low numbers (less than 8 eggs/g) were recovered from two animals (Table 3). By necropsy all/

Table 3: Recovery of S. mattheei eggs from the faeces (eggs/g)

Group	Sheep No.	Weeks After Challenge					
		$3\frac{1}{2}$	$5\frac{1}{2}$	$7\frac{1}{2}$	$9\frac{1}{2}$	$11\frac{1}{2}$	$13\frac{1}{2}$
Vaccinates	7	0	0	0	10	13	3
	52	0	0	0	0	0	5
	60	0	0	8	10	10	18
	19	0	0	2	5	13	10
	Mean	0	0	3	6	9	9
	S.E.	0	0	2	2	3	3
Non-Vaccinates	11	0	0	43	65	208	20
	95	0	0	93	N.D.	78	78
	68	0	0	58	35	53	50
	190	0	0	23	53	13	80
	Mean	0	0	54	51	88	57
	S.E.	0	0	15	9	42	14

N.D. Not Determined

all animals in this group were positive but the largest count was only 18 eggs/g, with a mean of 9 eggs/g. Based on measurements of total faecal output during the periods of confinement in metabolism cages, animals in this group excreted on average about 0.5 million eggs during patency. In contrast, all unvaccinated sheep were positive by $7\frac{1}{2}$ weeks with counts ranging from 23 to 93 eggs/g (mean 54 eggs/g), but no further increase was recorded subsequently and at necropsy the average count was 57 eggs/g, representing a total output of approximately 5 million eggs between the onset of oviposition and necropsy.

The results of the adult worm recoveries and tissue egg counts are shown in Table 4. Worm burdens, worm pairs and total liver eggs were all reduced by about 25% in the immunised as compared with the challenge control sheep and although none of these differences were statistically significant, when account was taken of the residual worm burden from the primary infection (based on the worm recovery of sheep No.10 which was necropsied 80 weeks after exposure to the hamster strain), worm recovery in the vaccinated group was significantly lower ($P < 0.02$). However by far the major difference between the groups concerned the intestinal egg counts which compared with the challenge controls were reduced respectively by 40% ($P > 0.05$) and 60% ($P < 0.003$) in the small and large intestines of the vaccinated sheep. The number of eggs recovered from the lungs was small in both groups but 50% lower in the vaccinated animals; total tissue egg counts were likewise substantially reduced in this group ($P < 0.05$) relative to the challenge controls.

Table 4: Worm Burdens and Tissue Egg Counts

Group	Sheep No.	Individual Worm Burdens	Individual Total Tissue Egg Counts ($\times 10^6$)	Total Tissue Eggs/Worm Pair ($\times 10^3$)	Distribution of Tissue Eggs (%)			
					Liver	Small Intestine	Large Intestine	Lungs
Vaccinates	7	2789	47.1	39.9	7.4	74.1	18.5	0.0
	52	2004	30.8	36.0	6.3	81.9	11.5	0.3
	60	2350	46.4	42.4	3.9	83.6	12.4	0.1
	19	2113	10.0	12.3	1.8	73.0	24.2	1.0
	Mean	2314	33.6	32.7	4.9	78.2	16.7	0.4
	S.E.	174	8.7	6.9	1.2	2.7	3.0	0.0
Non-Vaccinates	11	3477	91.6	58.6	5.0	81.8	13.1	0.1
	95	2793	44.1	36.8	4.6	68.9	26.5	0.0
	68	2863	47.0	38.1	1.4	70.3	28.2	0.1
	190	2478	60.9	56.8	4.8	70.6	24.6	0.0
	Mean	2903	60.9	47.6	4.0	72.9	23.1	0.1
	S.E.	209	10.9	5.9	0.8	3.0	3.4	0.0
Primary Infection	10	222	0.2	20.2	6.1	59.7	15.6	18.6

DISCUSSION

In the present experiment marked reductions in the clinical and pathophysiological effects of infection with a virulent strain of S. mattheei were induced in sheep by prior exposure to a strain of the parasite attenuated by hamster passage.

The non-vaccinated sheep exhibited an acute ovine schistosomiasis similar to that found by other workers [16-20], who found that the most severe clinico-pathophysiological changes were associated with the onset of oviposition at six weeks with a marked anaemia, hypo-albuminaemia and hypergammaglobulinaemia coinciding with the passage of soft blood-stained faeces and progressive inappetence. Similarly, in the non-vaccinates of the present experiment, most of the changes developed rapidly six weeks after challenge and coincided with the appearance of eggs in the faeces. However, the vaccinates differed from the non-vaccinates in several aspects of their clinico-pathophysiology, one of the most obvious differences being the earlier rise in globulin levels of the former. On the other hand, most of the clinico-pathological changes in these animals were generally slower to develop and milder in degree (Figs. 1 and 2) and these effects were reflected in the pathophysiological parameters measured prior to necropsy (Tables 1 and 2).

The protective effects of vaccination were due partly to a reduction in the worm burden resulting from challenge but mainly to a reduction in fecundity of the challenge population. Whilst similar manifestations of acquired immunity have been reported in bovine schistosomiasis/

schistosomiasis [6], the only previous study on the ability of sheep to resist reinfection with normal S. mattheei cercariae was carried out by Preston and Webbe [21]. They showed that pre-exposure to 500 S. mattheei cercariae produced no significant reduction either in worm burdens or egg counts resulting from challenge with 5,000 cercariae. Nevertheless, sheep are known to be capable of developing some immunity against this parasite. Preston and his co-workers [11] found that, although prior infection with normal S. mansoni cercariae did not significantly reduce the number of worms which established from a challenge of 10,000 S. mattheei cercariae, tissue egg loads were reduced by approximately 40%. However, immunisation with irradiated S. mattheei cercariae or schistosomula has been shown to be a more effective procedure. Taylor and his colleagues [12], compared the effectiveness of irradiated homologous and normal heterologous S. mansoni cercariae in sheep and found that, whereas the irradiated vaccine brought about reductions of 75% in worm and egg counts, the heterologous vaccine produced no significant immunity. The present report is of interest in that this is the first record of protective immunity being induced in schistosomiasis with a strain which has been attenuated by passage in laboratory animals.

SUMMARY

A study was carried out to establish whether previous exposure of sheep to a strain of S. mattheei, apparently attenuated by passage in hamsters, would afford any protection to the effects of a challenge infection of a virulent strain of the same parasite.

Four sheep were each infected with 10,000 cercariae of the avirulent strain and together with four worm-free sheep challenged 63 weeks later with 10,000 S. mattheei cercariae of a pathogenic strain. Four more sheep acted as uninfected controls. Following challenge the disease was monitored using clinical, pathophysiological and parasitological techniques.

The unvaccinated sheep developed severe disease 6-12 weeks after exposure characterised by marked anaemia, hypoalbuminaemia and hyperglobulinaemia coinciding with the passage of blood-stained faeces and progressive inappetence. In the vaccinated sheep, there was an even earlier rise in serum globulin levels, but the other clinico-pathophysiological changes were generally slower to develop and much milder in severity. The parasitological data showed that although this was partly due to a reduction in the establishment of the challenge worm population the main factor was probably a reduction in the fecundity of these worms.

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GENERAL DISCUSSION

The experiments described in this thesis have provided a considerable amount of information on some of the factors which affect the pathogenesis of parasitic infections in sheep. The mechanisms by which these factors operate have also been elucidated to a large extent, primarily as a result of the application of radioisotopic techniques. The findings were discussed in detail at the end of each chapter and it is the purpose of this final discussion to make a more general appraisal of some of the observations.

While the results of these present studies undoubtedly extend our knowledge of parasitic diseases it must be remembered that they were obtained by the application of strict experimental conditions and thus it is not necessarily valid to interpret them directly into the field situation.

Under natural conditions animals become infected with a parasite by picking up the infective stages over a period of days, months or even years. Although this can be simulated to a certain extent under experimental conditions by using a "trickle" infection, such a system leads to a complication of symptoms of the disease attributed to different stages of the parasites development in the host. In view of this latter feature, infection of animals by a single exposure is most commonly used in parasitic studies and was the method of choice in these present experiments. In this connection, the finding that the level of protein feeding had no effect upon the number of liver flukes which became established in the host may be open to question, since, immunological mechanisms stimulated by the presence/

presence of the parasite but requiring time to come into operation may not have been fairly tested by using a single dose of infective material. However, since sheep exhibit very little ability to "acquire" resistance to F. hepatica it is unlikely that the results would have been affected significantly by using "trickle" infections.

Perhaps the most abnormal condition it was necessary to enforce upon the animals in these studies was their maintenance in metabolic cages. Under such circumstances the sheep were not only sheltered from climatic stresses such as rain, wind and extreme temperature changes but also did not have to walk the long distances covered in the course of normal feeding. This latter feature would certainly be of particular importance to animals which are anaemic and thus more easily fatigued. It is therefore reasonable to assume that the effects of the parasite burdens encountered in these present studies would have been exacerbated under natural conditions because of increased energy requirements.

Despite this fact it was interesting to observe that sheep could harbour 200 adult liver flukes and provided with a high protein intake not only show very mild anaemia and hypoalbuminaemia but also gain weight at a rate comparable to worm-free sheep. The isotopic techniques revealed considerable enteric haemorrhage in these parasitised sheep and although the animals were well able to replace a lot of the destroyed red cells and plasma proteins, these losses nevertheless must represent an overall loss of metabolizable/

metabolizable energy. The ability of infected sheep to gain weight at a rate similar to worm-free animals must therefore be explained by one of two facts. Namely, the parasitised animals were able to extract more energy from the same dietary intake as worm-free sheep or differences in the total body energy of the parasitised compared to worm-free animals were taking place, but because of changes in the relative proportions of body components this was not reflected in body weight measurements. The findings of the present studies would certainly tend to support the latter theory but are far from conclusive and clearly, more accurate methods of measuring changes in body composition than the traditional balance techniques are required to establish the possible significance of this feature.

The important relationship between ovine fascioliasis and hostnutrition is clearly evident from the results of these present studies, and most certainly underline the importance of utilising anthelmintic treatments when animals enter periods of nutritional stress. Sheep under favourable conditions may tolerate quite high numbers of liver flukes without any apparent discomfort but when forage becomes short or of poorer quality or the demands of pregnancy and lactation start to drain the body stores, the same sheep may rapidly deteriorate into a clinical case of fascioliasis.

The most dramatic feature of the studies of ovine schistosomiasis was the loss of pathogenicity to sheep of a strain of S. mattheei which had been passaged through hamsters. The necessity/

necessity of most parasitological studies for a steady supply of large quantities of infective material is often met by the establishment of controlled cultural techniques which, because of economic factors and also ease of maintenance, often utilise laboratory animals as primary hosts. These present studies highlight the necessity of workers to be aware of possible changes to the parasite as a result of its passage through an abnormal host. The studies also indicate the importance of the parameters chosen to gauge the extent of the disease process. Clearly in respect to schistosomiasis the number of worms and/or parasite eggs within the hosts' tissues give no indication of the severity of disease in terms of the factors which are of most importance to the farmer, namely mortality and morbidity.

The finding in these studies of significant acquired resistance by sheep not primarily to the establishment of S. mattheei but rather to the pathogenic effects of infection may indicate that although the incidence of infected animals in the field may be high the incidence of actual clinical disease may be low.

APPENDIX 1

Data relevant to Chapter 1:

The Relationship of Host Nutrition and Fascioliasis:

The clinico-pathological changes in sheep following infection with F. hepatica

Appendix 1: Table 1

Experiment 1: Packed Cell Volume (%) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	25.5	29.8	25.8	27.0 ± 1.4	29.3	26.8	29.0	28.4 ± 0.8	24.5	28.3
1	25.3	31.0	25.8	27.4 ± 1.8	24.3	26.0	26.8	25.7 ± 0.7	23.3	26.8
2	27.5	28.3	28.0	27.9 ± 0.2	23.5	26.0	27.0	25.5 ± 1.0	24.5	25.5
3	26.8	26.0	29.3	27.4 ± 1.0	25.3	24.5	27.0	25.6 ± 0.7	29.0	25.5
4	27.3	23.5	28.8	26.5 ± 1.6	26.0	23.5	21.5	23.7 ± 1.3	28.0	26.5
5	28.1	24.2	28.4	26.9 ± 1.4	19.7	21.5	23.2	21.5 ± 1.0	26.9	28.9
6	26.8	22.6	27.5	25.6 ± 1.5	18.8	21.6	22.9	21.1 ± 1.2	24.2	28.0
7	26.9	22.4	27.4	25.6 ± 1.6	20.0	20.0	21.0	20.3 ± 0.3	22.9	25.5
8	26.4	22.9	27.1	25.5 ± 1.3	19.6	19.7	20.6	20.0 ± 0.3	21.9	23.6
9	26.6	23.3	27.8	25.9 ± 1.3	17.9	18.9	20.4	19.1 ± 0.7	22.5	21.8
10	27.7	21.6	26.5	25.3 ± 1.9	16.2	17.7	17.9	17.3 ± 0.5	22.1	19.3
11	26.9	22.7	27.0	25.5 ± 1.4	18.0	16.0	15.2	16.4 ± 0.8	16.9	19.6
12	26.5	20.5	26.4	24.5 ± 2.0	16.3	15.8	14.7	15.6 ± 0.5	14.2	19.5
13	26.8	20.3	25.3	24.1 ± 2.0	12.9	14.4	14.2	13.8 ± 0.5	12.9	17.2
14	27.7	23.2	24.5	25.1 ± 1.3	9.3	11.7	13.9	11.6 ± 1.3	12.0	14.2
15	27.3	22.3	23.7	24.4 ± 1.5	6.8	7.4	10.4	8.2 ± 1.1	10.1	11.2

+ Infected sheep without pair-fed control partners

Appendix 1: Table 2

Experiment 1: Packed Cell Volume (%) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	29.5	29.0	26.3	28.3 ± 1.0	31.5	26.8	26.3	28.2 ± 1.7	29.3	29.0
1	28.0	28.3	28.5	28.3 ± 0.1	29.3	23.5	26.8	26.5 ± 1.7	28.0	25.3
2	29.8	27.0	29.3	28.7 ± 0.9	30.5	24.5	25.8	26.9 ± 1.8	28.5	27.8
3	28.5	27.0	27.8	27.8 ± 0.4	33.0	24.5	26.0	27.8 ± 2.6	27.8	28.5
4	28.3	26.3	28.3	27.6 ± 0.7	32.0	25.8	27.5	28.4 ± 1.8	25.0	26.8
5	29.6	27.2	28.5	28.4 ± 0.7	30.1	25.6	26.1	27.3 ± 1.4	23.0	26.0
6	29.1	26.8	28.4	28.1 ± 0.7	28.2	24.5	26.2	26.3 ± 1.1	23.1	25.1
7	28.9	26.5	29.2	28.2 ± 0.9	27.9	24.2	24.9	25.7 ± 1.1	22.7	25.0
8	27.7	25.6	27.8	27.0 ± 0.7	26.4	22.4	22.1	23.6 ± 1.4	22.4	23.9
9	27.6	25.7	27.5	26.9 ± 0.6	26.0	20.3	21.1	22.5 ± 1.8	21.5	22.8
10	27.8	26.7	27.8	27.4 ± 0.4	24.6	17.7	19.0	20.4 ± 2.1	19.4	20.5
11	27.7	25.7	27.9	27.1 ± 0.7	22.5	17.1	17.4	19.0 ± 1.8	18.5	17.9
12	26.9	25.7	27.4	26.7 ± 0.5	21.1	17.2	15.3	17.9 ± 1.7	17.9	16.9
13	27.1	26.8	27.7	27.2 ± 0.3	21.9	16.6	13.5	17.3 ± 2.5	16.6	16.9
14	26.8	25.5	26.0	26.1 ± 0.4	22.8	15.5	14.0	17.4 ± 2.7	16.8	16.8
15	26.7	26.4	25.6	26.2 ± 0.3	20.3	14.6	15.2	16.7 ± 1.8	15.7	14.5
16	26.9	26.1	25.6	26.2 ± 0.4	19.6	13.7	14.5	15.9 ± 1.8	15.6	14.8
17	26.4	26.2	26.2	26.3 ± 0.1	18.2	10.7	13.6	14.2 ± 2.2	14.9	12.9
18	26.4	Dead	25.1	25.8 ± 0.7	16.7	Dead	11.9	14.3 ± 2.4	15.1	12.4
19	26.4		25.3	25.9 ± 0.6	16.5		10.8	13.7 ± 2.9	14.8	11.2
20	27.5		25.9	26.7 ± 0.8	16.1		11.3	13.7 ± 2.4	14.2	9.6

+ Infected sheep without pair-fed control partners

Appendix 1: Table 3

Experiment 1: Haemoglobin Concentration (g/100ml) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	8.02	9.56	7.94	8.51 ± 0.53	10.55	7.98	9.38	9.30 ± 0.74	7.10	8.86
1	8.35	10.08	8.30	8.91 ± 0.59	8.77	8.11	9.05	8.64 ± 0.28	7.34	9.19
2	8.81	9.16	8.77	8.91 ± 0.12	7.95	8.02	8.75	8.24 ± 0.26	7.94	8.33
3	8.74	8.26	9.03	8.68 ± 0.22	8.64	8.11	8.64	8.46 ± 0.18	9.64	8.79
4	8.94	8.04	9.23	8.74 ± 0.36	8.37	7.50	6.97	7.61 ± 0.41	9.32	8.68
5	9.43	8.11	9.18	8.91 ± 0.40	6.57	6.81	7.26	6.88 ± 0.20	8.66	9.63
6	9.26	7.61	9.07	8.65 ± 0.52	6.68	6.90	7.06	6.88 ± 0.11	7.95	8.92
7	9.54	7.69	8.99	8.74 ± 0.55	6.57	6.53	6.91	6.67 ± 0.12	7.58	8.31
8	9.05	7.80	8.90	8.58 ± 0.39	6.11	6.29	6.66	6.35 ± 0.16	7.21	7.80
9	8.99	7.59	9.19	8.59 ± 0.50	5.87	6.20	6.64	6.24 ± 0.22	7.30	7.12
10	9.32	7.32	8.50	8.38 ± 0.58	5.28	5.42	5.48	5.39 ± 0.06	6.71	6.25
11	8.72	7.39	8.55	8.22 ± 0.42	5.54	4.78	4.82	5.05 ± 0.25	5.34	6.11
12	9.20	7.32	9.19	8.57 ± 0.63	4.76	4.78	4.82	4.79 ± 0.02	4.69	6.05
13	9.08	6.99	7.74	7.94 ± 0.61	3.40	3.99	4.43	3.94 ± 0.30	3.96	5.08
14	8.83	7.61	7.17	7.87 ± 0.50	2.28	2.82	3.98	3.03 ± 0.50	3.68	4.01
15		8.33	8.28	8.31 ± 0.02		1.88	2.91	2.40 ± 0.51	2.50	4.47

+ Infected sheep without pair-fed control partners

Appendix 1: Table 4

Experiment 1: Haemoglobin Concentration (g/100ml) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	8.75	9.01	7.57	8.44 ± 0.44	9.27	7.91	8.16	8.45 ± 0.72	9.19	9.30
1	9.09	9.44	8.96	9.16 ± 0.14	9.32	8.07	7.82	8.40 ± 0.46	9.50	8.35
2	9.34	8.88	8.90	9.04 ± 0.15	9.49	8.06	8.28	8.61 ± 0.44	9.23	8.71
3	8.86	8.55	9.60	9.00 ± 0.31	10.21	7.69	8.60	8.83 ± 0.74	9.62	8.99
4	8.99	8.79	8.98	8.92 ± 0.07	10.22	8.53	9.05	9.27 ± 0.50	8.16	-9.91
5	9.75	9.50	9.12	9.46 ± 0.18	9.51	8.76	8.75	9.01 ± 0.25	7.80	7.85
6	9.71	9.25	9.41	9.46 ± 0.13	9.12	8.37	9.05	8.85 ± 0.24	7.92	8.28
7	9.58	9.03	9.42	9.34 ± 0.16	9.01	8.08	8.20	8.43 ± 0.29	7.67	8.24
8	9.05	8.57	9.25	8.96 ± 0.20	8.96	7.41	7.38	7.92 ± 0.52	7.50	8.02
9	9.10	8.63	8.94	8.89 ± 0.14	8.75	6.73	7.13	7.54 ± 0.62	7.26	7.54
10	9.01	9.05	9.38	9.15 ± 0.12	7.78	5.61	6.44	6.61 ± 0.63	6.47	6.68
11	8.64	8.64	9.05	8.78 ± 0.14	7.26	5.29	5.61	6.05 ± 0.61	5.98	5.59
12	9.03	8.85	9.41	9.10 ± 0.17	7.21	5.57	4.80	5.86 ± 0.71	6.11	5.52
13	9.06	9.58	9.16	9.27 ± 0.16	8.07	5.02	4.74	5.94 ± 1.07	5.52	5.50
14	8.88	8.79	8.79	8.82 ± 0.03	6.92	4.71	4.67	5.43 ± 0.74	5.28	4.86
15	8.84	9.91	8.90	9.22 ± 0.35	6.58	4.60	5.48	5.55 ± 0.57	5.07	4.53
16	8.33	8.64	8.46	8.48 ± 0.09	5.76	4.10	4.58	4.81 ± 0.49	5.06	4.54
17	8.66	9.05	8.42	8.71 ± 0.18	5.41	3.09	4.23	4.24 ± 0.67	4.58	3.70
18	8.68	Dead	7.93	8.31 ± 0.37	5.11	Dead	3.60	4.36 ± 0.75	4.56	3.74
19	8.60		7.76	8.18 ± 0.42	5.04		3.16	4.10 ± 0.94	4.27	2.90
20	9.01		8.27	8.64 ± 0.37	5.04		2.42	3.73 ± 1.31	3.79	2.50

+ Infected sheep without pair-fed control partners

Appendix 1: Table 5

Experiment 1: Red Cell Counts (millions/mm^3) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	6.32	8.56	6.42	7.10 ± 0.73	8.66	7.00	7.66	7.77 ± 0.48	6.72	7.48
1	6.42	8.99	6.78	7.40 ± 0.80	7.41	6.86	7.43	7.23 ± 0.19	6.08	7.07
2	7.17	7.29	7.32	7.26 ± 0.05	6.76	7.33	7.05	7.05 ± 0.16	6.40	6.98
3	6.77	7.40	6.99	7.05 ± 0.18	6.88	6.75	7.00	6.88 ± 0.07	7.67	6.82
4	7.02	7.01	7.30	7.11 ± 0.09	4.78	6.64	6.61	6.01 ± 0.61	8.00	7.51
5	7.35	6.85	7.21	7.14 ± 0.15	4.18	5.99	6.59	5.59 ± 0.72	7.14	8.30
6	7.35	6.84	7.33	7.09 ± 0.24	4.47	5.87	6.14	5.49 ± 0.52	6.39	8.21
7	7.31	6.43	6.83	6.86 ± 0.25	5.03	5.40	5.93	5.45 ± 0.26	5.99	6.89
8	7.23	6.71	7.39	7.11 ± 0.21	5.72	5.66	5.99	5.79 ± 0.10	6.25	6.86
9	7.51	6.94	7.47	7.31 ± 0.18	4.81	5.74	5.79	5.45 ± 0.32	6.68	6.23
10	7.72	6.51	7.06	7.10 ± 0.35	4.72	5.11	5.29	5.04 ± 0.17	6.18	5.78
11	7.24	6.46	7.10	6.93 ± 0.24	4.79	4.54	4.38	4.57 ± 0.12	4.75	5.17
12	7.31	6.17	7.36	6.95 ± 0.39	4.16	5.11	3.89	4.39 ± 0.37	3.98	4.86
13	7.38	5.84	6.54	6.59 ± 0.45	3.80	3.32	3.64	3.59 ± 0.14	3.52	4.02
14	6.99	6.63	6.03	6.55 ± 0.28	2.67	2.59	3.24	2.83 ± 0.24	3.05	3.90
15		7.72	6.75	7.24 ± 0.48		2.31	2.71	2.51 ± 0.20	2.10	3.37

+ Infected sheep without pair-fed control partners

Appendix 1: Table 6

Experiment 1: Red Cell Counts (millions/mm^3) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	7.84	8.00	7.04	7.63 ± 0.30	8.26	7.04	6.34	7.21 ± 0.56	7.48	8.06
1	7.52	7.48	7.37	7.46 ± 0.04	6.61	7.33	6.87	6.94 ± 0.21	7.43	7.13
2	7.76	7.62	7.86	7.75 ± 0.07	7.62	6.73	7.19	7.18 ± 0.26	7.50	7.48
3	7.66	7.53	7.66	7.62 ± 0.04	8.74	7.03	7.57	7.78 ± 0.50	7.67	8.18
4	7.93	7.73	8.04	7.90 ± 0.09	8.78	7.38	8.09	8.08 ± 0.40	6.80	8.09
5	8.40	8.30	7.98	8.23 ± 0.13	8.14	7.86	7.93	7.98 ± 0.08	6.56	7.65
6	7.31	8.17	8.45	7.98 ± 0.34	8.26	7.28	7.64	7.73 ± 0.29	6.64	7.36
7	8.04	7.30	8.05	7.80 ± 0.25	7.24	6.60	6.97	6.94 ± 0.19	6.53	6.89
8	8.31	7.53	8.13	7.99 ± 0.24	7.49	6.47	6.50	6.82 ± 0.34	6.27	7.82
9	8.10	7.58	8.00	7.89 ± 0.16	7.44	6.11	6.50	6.68 ± 0.39	6.51	6.94
10	8.96	8.41	8.54	8.64 ± 0.17	7.13	5.18	5.80	6.04 ± 0.58	5.88	6.11
11	8.09	8.08	8.65	8.27 ± 0.19	6.65	4.37	5.61	5.54 ± 0.66	5.34	5.40
12	7.68	7.82	8.33	7.94 ± 0.20	5.29	4.04	4.25	4.53 ± 0.39	5.47	4.81
13	8.30	9.86	8.70	8.95 ± 0.47	6.62	3.70	4.00	4.77 ± 0.93	4.78	5.66
14	8.21	8.26	8.17	8.21 ± 0.02	6.12	3.47	4.56	4.72 ± 0.77	5.49	4.49
15	6.86	8.97	8.23	8.02 ± 0.62	5.41	3.23	5.35	4.66 ± 0.72	4.94	3.74
16	7.40	7.90	7.71	7.67 ± 0.15	4.56	2.96	4.11	3.88 ± 0.48	4.45	3.39
17	7.66	8.22	7.78	7.89 ± 0.17	4.09	2.51	3.86	3.49 ± 0.49	3.84	2.99
18	7.78	Dead	7.28	7.53 ± 0.25	3.68	Dead	3.58	3.63 ± 0.05	3.71	3.45
19	7.92		7.36	7.64 ± 0.28	3.60		2.94	3.27 ± 0.33	3.54	2.38
20	8.90		8.40	8.65 ± 0.25	3.62		2.30	2.96 ± 0.66	3.40	2.30

+ Infected sheep without pair-fed control partners

Appendix 1: Table 7

Experiment 1: Mean Corpuscular Volume (μm^3) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	39.6	34.5	39.7	37.9 ± 1.7	36.4	36.4	35.9	36.2 ± 1.7	34.2	37.4
1	39.3	34.5	38.0	37.3 ± 1.4	33.0	37.9	36.0	35.6 ± 1.4	38.2	37.8
2	38.4	38.9	38.3	38.5 ± 0.2	34.8	35.5	38.3	35.4 ± 1.1	38.3	36.6
3	39.5	35.1	41.8	38.8 ± 2.0	36.7	36.3	36.2	36.4 ± 0.2	37.8	37.4
4	38.8	33.5	39.4	37.2 ± 1.9	45.0	35.4	36.2	38.9 ± 3.1	35.0	35.3
5	37.8	35.0	38.5	37.1 ± 1.1	47.3	35.1	34.9	39.1 ± 4.1	37.1	35.0
6	37.5	32.9	37.2	35.9 ± 1.5	43.0	36.2	36.2	38.5 ± 2.3	37.2	32.9
7	38.3	35.4	39.6	37.8 ± 1.2	39.4	37.5	35.0	37.3 ± 1.3	38.5	36.3
8	36.0	32.8	36.9	35.2 ± 1.3	33.7	33.1	35.1	34.0 ± 0.6	35.2	34.6
9	37.3	33.2	37.2	35.9 ± 1.4	36.4	33.1	34.5	34.7 ± 1.0	34.1	34.5
10	36.0	33.4	36.8	35.4 ± 1.0	32.9	33.3	32.6	32.9 ± 0.2	34.6	33.0
11	35.9	33.3	36.6	35.3 ± 1.0	36.5	33.6	34.2	34.8 ± 0.9	34.2	36.8
12	35.6	34.0	37.7	35.8 ± 1.1	37.3	32.6	36.8	35.6 ± 1.5	35.2	38.1
13	35.9	32.8	35.2	34.6 ± 0.9	31.6	40.7	38.5	36.9 ± 2.7	35.5	42.3
14	38.8	33.3	37.7	36.6 ± 1.7	30.0	38.4	39.2	35.9 ± 2.9	38.4	39.0
15		32.4	38.6	35.5 ± 3.1		31.4	38.7	35.1 ± 3.7	42.9	36.9

+ Infected sheep without pair-fed control partners

Appendix 1: Table 8

Experiment 1: Mean Corpuscular Volume (μm^3) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	35.7	35.0	36.9	35.9 ± 0.5	37.5	36.2	39.4	37.7 ± 0.9	38.8	34.7
1	37.2	38.2	38.6	38.0 ± 0.4	44.7	32.3	39.4	38.8 ± 3.6	37.7	35.5
2	38.4	35.5	37.3	37.1 ± 0.8	40.3	36.4	35.8	37.5 ± 1.4	38.0	37.1
3	37.3	35.9	36.2	36.5 ± 0.4	37.8	34.8	34.4	35.7 ± 1.1	36.2	34.8
4	35.6	34.0	35.2	34.9 ± 0.5	36.5	34.9	34.2	35.2 ± 0.7	36.8	33.0
5	35.8	33.8	37.8	35.8 ± 1.2	36.5	33.0	33.2	34.2 ± 1.1	35.1	35.3
6	35.5	32.7	34.1	34.1 ± 0.8	33.9	33.0	35.9	34.3 ± 0.9	34.6	34.3
7	36.4	37.0	36.4	36.6 ± 0.2	38.7	37.1	35.5	37.1 ± 0.9	35.2	36.3
8	33.4	34.2	33.9	33.8 ± 0.2	36.0	34.0	33.1	34.4 ± 0.9	34.7	30.1
9	33.7	33.3	33.8	33.6 ± 0.2	34.3	32.8	31.9	33.0 ± 0.7	33.4	32.4
10	32.4	32.1	32.5	32.3 ± 0.1	34.0	33.4	32.8	33.4 ± 0.3	33.3	32.8
11	32.5	31.3	31.9	31.9 ± 0.3	33.1	38.9	30.3	34.1 ± 2.5	34.2	32.0
12	34.5	33.3	33.7	33.8 ± 0.4	40.8	42.7	33.5	39.0 ± 2.8	32.5	34.4
13	33.7	27.4	32.2	31.1 ± 1.9	34.7	43.2	33.8	37.2 ± 3.0	35.6	31.8
14	32.9	30.9	32.7	32.2 ± 0.6	35.9	44.7	31.8	37.5 ± 3.8	29.1	34.0
15	42.5	31.9	33.0	35.8 ± 3.4	40.7	48.8	31.6	40.4 ± 5.0	33.9	40.3
16	34.2	32.0	34.7	33.6 ± 0.8	40.6	46.4	35.3	40.8 ± 3.2	36.5	42.8
17	34.9	32.6	33.5	33.7 ± 0.7	44.0	39.6	33.7	39.1 ± 3.0	37.8	40.1
18	34.0	Dead	33.7	33.9 ± 0.2	46.3	Dead	31.9	39.1 ± 7.2	43.2	38.4
19	32.8		32.6	32.7 ± 0.1	44.4		39.1	41.8 ± 2.7	38.1	44.1
20	29.8		29.8	29.8 ± 0.0	41.4		39.1	40.3 ± 1.2	41.2	39.1

+ Infected sheep without pair-fed control partners

Appendix 1: Table 9

Experiment 1: Mean Corpuscular Haemoglobin Concentration (%) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	32.1	32.4	31.1	31.9 ± 0.4	33.5	31.3	34.1	33.0 ± 0.9	30.9	31.6
1	33.1	32.5	32.2	32.6 ± 0.3	36.2	31.2	33.8	33.7 ± 1.4	31.5	34.3
2	32.0	32.4	31.3	31.9 ± 0.3	33.8	30.8	32.4	32.3 ± 0.9	32.4	32.7
3	32.7	31.8	30.9	31.8 ± 0.5	34.2	33.2	32.0	33.1 ± 0.6	33.2	34.5
4	32.8	34.2	32.1	33.0 ± 0.6	32.2	31.9	32.4	32.2 ± 0.2	33.3	32.7
5	34.1	33.8	33.1	33.7 ± 0.3	33.3	32.4	31.9	32.5 ± 0.4	32.7	33.2
6	33.7	33.8	33.3	33.6 ± 0.2	34.8	32.4	31.7	33.0 ± 0.9	33.5	33.0
7	34.1	33.8	33.3	33.7 ± 0.2	33.2	32.2	33.3	32.9 ± 0.4	33.0	33.2
8	32.9	33.9	32.6	33.1 ± 0.4	32.1	32.7	32.5	32.4 ± 0.2	32.4	33.2
9	32.1	33.1	33.1	32.8 ± 0.3	33.5	32.6	33.1	33.1 ± 0.3	32.1	33.1
10	33.6	33.7	32.7	33.3 ± 0.3	33.0	31.4	32.2	32.2 ± 0.5	30.8	32.5
11	33.5	34.4	32.9	33.6 ± 0.4	31.6	31.3	32.1	31.7 ± 0.2	32.8	32.2
12	35.4	34.9	33.1	34.5 ± 0.7	30.7	31.9	33.8	32.1 ± 0.9	33.5	32.7
13	34.9	34.9	33.7	34.5 ± 0.4	30.8	30.1	32.8	31.2 ± 0.8	33.6	32.1
14	32.8	34.6	31.5	33.0 ± 0.9	28.3	28.0	31.2	29.2 ± 1.0	31.3	31.4
15		33.3	31.8	32.6 ± 0.8		25.8	27.8	26.8 ± 1.0	27.8	34.8

+ Infected sheep without pair-fed control partners

Appendix 1: Table 10

Experiment 1: Mean Corpuscular Haemoglobin Concentration (%) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	31.3	32.2	29.1	30.9 ± 0.9	29.9	31.0	32.6	31.2 ± 0.7	31.7	33.2
1	32.4	33.5	31.5	32.5 ± 0.6	31.9	34.3	29.4	31.9 ± 1.4	33.9	33.1
2	31.4	32.9	30.4	31.6 ± 0.7	31.1	32.9	32.1	32.0 ± 0.5	32.4	31.4
3	31.1	31.7	34.7	32.5 ± 1.1	30.9	31.4	33.2	31.8 ± 0.7	34.7	31.5
4	31.8	33.5	31.8	32.4 ± 0.6	31.9	33.1	32.9	32.6 ± 0.4	32.6	37.3
5	32.5	33.9	32.0	32.8 ± 0.6	32.0	34.0	33.3	33.1 ± 0.6	33.9	30.9
6	32.9	34.6	32.7	33.4 ± 0.6	32.6	34.9	32.9	33.5 ± 0.7	34.4	32.8
7	32.7	33.4	32.2	32.8 ± 0.3	32.2	33.0	33.1	32.8 ± 0.3	33.4	33.0
8	32.9	33.6	33.0	33.2 ± 0.2	33.5	34.1	33.5	33.7 ± 0.2	33.3	34.5
9	33.4	34.2	33.1	33.6 ± 0.3	34.3	33.7	34.4	34.1 ± 0.2	33.2	33.5
10	33.4	33.8	33.5	33.6 ± 0.1	32.4	32.5	34.3	33.1 ± 0.6	34.5	33.4
11	32.9	34.2	32.9	33.3 ± 0.4	33.0	31.1	33.0	32.4 ± 0.6	32.7	32.4
12	34.1	34.1	33.6	33.9 ± 0.1	33.9	32.4	33.7	33.3 ± 0.5	34.4	33.4
13	33.2	36.9	33.9	34.7 ± 1.1	35.5	32.4	35.1	34.3 ± 1.0	32.9	31.4
14	32.9	34.5	32.9	33.4 ± 0.5	31.4	30.3	32.2	31.3 ± 0.6	32.9	31.8
15	31.6	34.6	32.8	33.0 ± 0.9	30.0	29.2	32.4	30.5 ± 1.0	30.3	30.1
16	33.0	34.2	31.7	33.0 ± 0.7	31.1	29.8	31.6	30.8 ± 0.5	31.1	31.3
17	32.7	35.3	33.0	33.7 ± 0.8	31.0	29.9	33.2	31.4 ± 1.0	31.5	31.4
18	32.8	Dead	32.4	32.6 ± 0.2	30.1	Dead	31.9	31.0 ± 1.0	28.5	29.3
19	33.1		32.3	32.7 ± 0.4	31.5		27.5	29.5 ± 2.0	31.6	27.6
20	34.0		33.1	33.6 ± 0.5	33.6		26.9	30.3 ± 3.4	27.1	27.8

+ Infected sheep without pair-fed control partners

Appendix 1: Table 11

Experiment 1: Total Serum Protein Concentrations (g/100ml) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	5.21	5.29	5.74	5.41 ± 0.16	5.38	5.67	5.52	5.52 ± 0.08	6.02	5.51
1	5.16	4.96	5.32	5.15 ± 0.10	5.54	5.18	5.34	5.35 ± 0.10	5.40	4.63
2	5.23	5.07	5.54	5.28 ± 0.14	5.74	5.80	6.18	5.91 ± 0.14	6.00	5.36
3	5.07	4.74	5.60	5.14 ± 0.25	5.93	5.80	6.46	6.06 ± 0.20	6.22	5.43
4	5.09	4.98	5.37	5.15 ± 0.12	6.07	5.94	6.59	6.20 ± 0.20	7.07	5.50
5	5.14	4.83	5.34	5.10 ± 0.15	6.31	6.00	7.08	6.46 ± 0.32	7.21	5.96
6	4.92	4.74	5.58	5.08 ± 0.26	6.82	6.20	7.35	6.79 ± 0.33	7.61	6.00
7	5.16	4.92	5.56	5.21 ± 0.19	7.68	6.73	7.94	7.45 ± 0.37	6.97	5.96
8	5.16	5.12	5.51	5.26 ± 0.12	7.74	6.62	8.23	7.53 ± 0.48	7.24	5.74
9	5.12	4.94	5.21	5.09 ± 0.08	7.54	6.73	8.69	7.65 ± 0.57	6.84	5.38
10	4.99	4.94	5.32	5.08 ± 0.12	7.65	6.53	8.38	7.52 ± 0.54	6.95	5.16
11	5.36	5.10	5.29	5.25 ± 0.08	6.35	5.93	8.07	6.78 ± 0.65	6.78	4.61
12	4.99	5.12	4.79	4.97 ± 0.10	5.40	5.40	7.19	6.00 ± 0.60	6.51	4.08
13	4.81	4.90	4.87	4.86 ± 0.03	4.72	4.50	6.42	5.21 ± 0.61	6.84	3.62
14	4.90	5.10	5.05	5.02 ± 0.06	4.15	3.99	6.02	4.72 ± 0.65	6.44	3.44
15		5.16	5.14	5.15 ± 0.01		3.49	5.62	4.56 ± 1.07	5.01	3.40

+ Infected sheep without pair-fed control partners

Appendix 1: Table 12

Experiment 1: Total Serum Protein Concentrations (g/100ml) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	5.91	5.74	5.54	5.73 ± 0.11	6.09	6.29	5.89	6.09 ± 0.12	6.62	5.34
1	5.54	5.47	5.27	5.43 ± 0.08	5.58	5.58	5.67	5.61 ± 0.03	6.07	4.87
2	5.78	5.34	5.56	5.56 ± 0.13	6.13	6.00	5.96	6.03 ± 0.05	6.66	5.36
3	5.96	5.29	5.29	5.51 ± 0.22	6.22	5.87	5.93	6.01 ± 0.11	7.08	5.43
4	5.88	5.34	5.36	5.53 ± 0.18	6.55	6.07	6.15	6.26 ± 0.15	6.99	5.93
5	6.04	5.36	5.34	5.58 ± 0.23	7.15	6.07	6.24	6.49 ± 0.34	7.72	6.04
6	5.93	5.85	5.62	5.80 ± 0.09	7.26	6.04	6.46	6.59 ± 0.36	7.81	6.13
7	6.02	5.10	5.51	5.54 ± 0.27	7.01	6.31	6.95	6.76 ± 0.22	7.52	6.53
8	5.80	5.51	5.51	5.61 ± 0.10	6.95	6.31	6.51	6.59 ± 0.19	7.74	6.31
9	5.76	5.34	5.23	5.44 ± 0.16	7.06	6.51	6.71	6.76 ± 0.16	7.90	6.20
10	6.04	6.00	5.74	5.93 ± 0.09	7.80	6.42	7.24	7.15 ± 0.40	8.18	6.31
11	5.96	5.78	5.60	5.78 ± 0.10	7.32	5.65	5.21	6.06 ± 0.64	7.61	6.33
12	5.67	5.21	5.18	5.35 ± 0.16	6.93	4.76	6.73	6.14 ± 0.69	7.70	5.34
13	5.43	5.34	5.05	5.27 ± 0.11	6.84	3.99	6.73	5.85 ± 0.93	7.04	4.96
14	5.80	5.85	5.27	5.64 ± 0.19	6.97	3.99	6.88	5.95 ± 0.98	7.17	4.54
15	5.80	5.67	5.29	5.59 ± 0.15	5.74	2.89	6.15	4.93 ± 1.03	4.87	3.71
16	5.65	5.47	5.18	5.43 ± 0.14	5.85	2.74	5.56	4.72 ± 0.99	5.96	3.42
17	5.98	5.82	5.21	5.67 ± 0.23	5.60	2.43	5.56	4.53 ± 1.05	5.62	3.35
18	5.71	Dead	5.29	5.50 ± 0.21	4.30	Dead	4.43	4.37 ± 0.06	4.61	2.87
19	6.07		5.38	5.73 ± 0.34	4.32		4.17	4.25 ± 0.07	4.43	2.74
20	6.02		5.36	5.69 ± 0.33	3.97		3.64	3.81 ± 0.16	4.01	2.69

+ Infected sheep without pair-fed control partners

Appendix 1: Table 13

Experiment 1: Serum Albumin Concentrations (g/100ml) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	2.62	2.25	2.98	2.62 ± 0.21	2.46	2.54	2.47	2.49 ± 0.02	2.66	3.21
1	2.85	2.37	2.60	2.61 ± 0.14	2.73	2.38	2.30	2.47 ± 0.13	2.63	2.97
2	2.86	2.23	2.54	2.54 ± 0.18	2.61	2.38	2.34	2.44 ± 0.08	2.77	2.92
3	2.83	2.18	2.70	2.57 ± 0.20	2.60	2.21	2.19	2.33 ± 0.13	2.38	2.93
4	2.98	2.33	2.74	2.68 ± 0.19	2.35	1.94	2.27	2.19 ± 0.13	1.73	2.91
5	2.96	2.25	2.65	2.62 ± 0.21	2.39	2.13	2.25	2.26 ± 0.07	1.84	2.93
6	2.58	2.17	2.39	2.38 ± 0.12	2.27	1.78	2.09	2.05 ± 0.14	1.50	2.43
7	2.63	2.08	2.52	2.41 ± 0.17	1.81	1.86	1.62	1.76 ± 0.07	1.50	2.54
8	2.72	2.38	2.63	2.58 ± 0.10	1.83	1.59	1.34	1.59 ± 0.14	0.88	2.33
9	2.87	2.18	2.71	2.59 ± 0.21	1.59	1.59	1.28	1.49 ± 0.10	0.95	1.91
10	2.78	2.21	2.71	2.57 ± 0.18	1.41	1.43	0.91	1.25 ± 0.17	1.06	1.91
11	2.57	2.04	2.57	2.39 ± 0.18	1.11	1.27	0.83	1.07 ± 0.13	0.69	1.83
12	2.25	1.98	2.03	2.09 ± 0.08	0.92	1.08	0.72	0.91 ± 0.10	0.97	1.56
13	2.44	1.89	2.22	2.18 ± 0.16	0.84	0.61	0.77	0.74 ± 0.07	0.61	1.48
14	2.20	1.82	1.93	1.98 ± 0.11	0.73	0.74	0.81	0.76 ± 0.02	0.75	1.33
15		2.06	2.28	2.17 ± 0.11		0.51	0.84	0.68 ± 0.16	0.75	1.27

+ Infected sheep without pair-fed control partners

Appendix 1: Table 14

Experiment 1: Serum Albumin Concentrations (g/100ml) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	3.17	3.12	2.66	2.98 ± 0.16	3.02	3.00	2.65	2.89 ± 0.12	2.78	2.96
1	3.24	3.40	2.78	3.14 ± 0.19	3.14	2.86	2.74	2.91 ± 0.12	2.85	3.05
2	3.26	3.26	2.91	3.14 ± 0.12	2.99	3.05	2.81	2.95 ± 0.07	2.73	2.84
3	3.58	3.45	3.03	3.35 ± 0.17	2.70	3.00	2.79	2.83 ± 0.09	2.57	2.54
4	2.98	3.39	3.10	3.16 ± 0.12	2.35	2.98	2.68	2.67 ± 0.18	2.47	2.27
5	2.90	3.37	3.15	3.14 ± 0.14	2.50	2.96	2.66	2.71 ± 0.13	2.57	2.45
6	2.98	3.28	2.49	2.92 ± 0.23	2.25	2.63	1.97	2.28 ± 0.19	2.33	2.55
7	3.06	3.24	3.03	3.11 ± 0.07	2.35	2.83	2.02	2.40 ± 0.24	2.13	2.65
8	2.95	3.24	3.42	3.20 ± 0.14	2.55	2.48	2.33	2.45 ± 0.06	2.33	2.62
9	3.14	3.05	3.16	3.12 ± 0.03	2.13	2.03	2.10	2.09 ± 0.03	2.20	2.19
10	3.38	3.18	3.11	3.22 ± 0.08	1.84	2.17	1.63	1.88 ± 0.16	2.07	2.13
11	3.35	3.19	3.13	3.22 ± 0.07	1.43	1.86	1.03	1.44 ± 0.24	1.83	1.92
12	2.96	2.71	2.75	2.81 ± 0.08	1.20	1.41	1.35	1.32 ± 0.06	1.63	1.56
13	2.96	2.84	2.76	2.85 ± 0.06	1.20	1.01	1.15	1.12 ± 0.06	1.15	1.89
14	2.87	2.84	2.72	2.81 ± 0.05	1.06	1.15	0.94	1.05 ± 0.06	1.28	1.47
15	3.06	2.90	2.98	2.98 ± 0.05	1.51	1.20	1.40	1.37 ± 0.09	1.31	1.62
16	2.53	2.34	2.25	2.37 ± 0.08	1.17	0.57	0.92	0.89 ± 0.17	1.03	0.95
17	2.52	2.20	2.17	2.30 ± 0.11	1.14	0.36	0.95	0.82 ± 0.23	0.84	0.71
18	2.46	Dead	2.06	2.26 ± 0.20	0.74	Dead	0.63	0.69 ± 0.05	0.63	0.52
19	2.51		2.14	2.33 ± 0.18	0.84		0.45	0.65 ± 0.19	0.78	0.42
20	2.60		2.03	2.32 ± 0.28	0.62		0.44	0.53 ± 0.09	0.71	0.35

+ Infected sheep without pair-fed control partners

Appendix 1: Table 15

Experiment 1: Serum Globulin Concentrations (g/100ml) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	2.59	3.04	2.76	2.80 ± 0.13	2.92	3.13	3.05	3.03 ± 0.06	3.36	2.30
1	2.31	2.59	2.72	2.54 ± 0.12	2.81	2.80	3.04	2.88 ± 0.08	2.77	1.66
2	2.37	2.84	3.00	2.74 ± 0.19	3.13	3.42	3.84	3.46 ± 0.21	3.23	2.44
3	2.24	2.56	2.90	2.57 ± 0.19	3.33	3.59	4.27	3.73 ± 0.28	3.84	2.50
4	2.12	2.70	2.64	2.49 ± 0.18	3.80	3.95	4.39	4.05 ± 0.18	5.79	2.60
5	2.18	2.58	2.69	2.48 ± 0.15	3.92	3.87	4.83	4.21 ± 0.31	5.37	3.03
6	2.34	2.57	3.19	2.70 ± 0.25	4.55	4.42	5.26	4.74 ± 0.26	6.11	3.57
7	2.53	2.84	3.04	2.80 ± 0.15	5.87	4.87	6.32	5.69 ± 0.43	5.47	3.42
8	2.44	2.74	2.88	2.69 ± 0.13	5.91	5.03	6.89	5.94 ± 0.54	6.36	3.41
9	2.25	2.76	2.50	2.50 ± 0.15	5.95	5.14	7.41	6.17 ± 0.66	5.89	3.47
10	2.21	2.73	2.61	2.52 ± 0.16	6.24	5.10	7.47	6.27 ± 0.68	5.89	3.25
11	2.79	3.06	2.72	2.86 ± 0.10	5.24	4.66	7.24	5.71 ± 0.78	6.09	2.78
12	2.74	3.14	2.76	2.88 ± 0.13	4.48	4.32	6.47	5.09 ± 0.69	5.54	2.52
13	2.37	3.01	2.65	2.68 ± 0.19	3.88	3.89	5.65	4.47 ± 0.59	6.23	2.14
14	2.70	3.28	3.12	3.03 ± 0.17	3.42	3.25	5.21	3.96 ± 0.63	5.69	2.11
15		3.10	2.86	2.98 ± 0.12		2.98	4.78	3.88 ± 0.90	4.26	2.13

+ Infected sheep without pair-fed control partners

Appendix 1: Table 16

Experiment 1: Serum Globulin Concentrations (g/100ml) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	2.74	2.62	2.88	2.75 ± 0.07	3.07	3.29	3.24	3.20 ± 0.07	3.84	2.38
1	2.30	2.07	2.49	2.29 ± 0.21	2.44	2.72	2.93	2.70 ± 0.14	3.22	1.82
2	2.52	2.08	2.65	2.42 ± 0.17	3.14	2.95	3.15	3.08 ± 0.06	3.93	2.52
3	2.38	1.84	2.26	2.16 ± 0.16	3.52	2.87	3.14	3.18 ± 0.19	4.51	2.89
4	2.91	1.95	2.26	2.37 ± 0.28	4.51	3.09	3.47	3.69 ± 0.42	4.52	3.66
5	3.14	1.99	2.19	2.44 ± 0.35	4.65	3.11	3.58	3.78 ± 0.46	5.15	3.59
6	2.95	2.57	3.13	2.88 ± 0.16	5.01	3.41	4.49	4.30 ± 0.47	5.48	3.58
7	2.96	1.86	2.48	2.43 ± 0.32	4.66	3.48	4.93	4.36 ± 0.45	5.39	3.88
8	2.85	2.27	2.09	2.40 ± 0.23	4.40	3.83	4.18	4.14 ± 0.17	5.41	3.69
9	2.62	2.29	2.07	2.33 ± 0.16	4.93	4.48	4.61	4.67 ± 0.13	5.70	4.01
10	2.66	2.82	2.63	2.70 ± 0.06	5.96	4.25	5.61	5.27 ± 0.52	6.11	4.18
11	2.61	2.59	2.47	2.56 ± 0.04	5.89	3.79	4.18	4.62 ± 0.64	5.78	4.41
12	2.71	2.50	2.43	2.55 ± 0.08	5.73	3.35	5.38	4.82 ± 0.74	6.07	3.78
13	2.47	2.50	2.29	2.42 ± 0.07	5.64	2.98	5.58	4.73 ± 0.88	5.89	3.07
14	2.93	3.01	2.55	2.83 ± 0.14	5.91	2.84	5.94	4.90 ± 1.03	5.89	3.07
15	2.81	2.92	2.58	2.77 ± 0.10	5.11	2.44	5.75	4.43 ± 1.01	5.69	2.88
16	3.12	3.13	2.93	3.06 ± 0.06	4.68	2.17	4.64	3.83 ± 0.83	4.93	2.47
17	3.46	3.62	3.04	3.37 ± 0.17	4.46	2.07	4.61	3.71 ± 0.82	4.78	2.64
18	3.25	Dead	3.23	3.24 ± 0.01	3.56	Dead	3.80	3.68 ± 0.12	3.98	2.35
19	3.56		3.24	3.40 ± 0.16	3.48		3.72	3.60 ± 0.12	3.65	2.32
20	3.42		3.33	3.38 ± 0.04	3.35		3.20	3.28 ± 0.07	3.30	2.34

+ Infected sheep without pair-fed control partners

Appendix 1: Table 17

Experiment 1: Serum Albumin:Globulin Ratios in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
0	1.01	0.74	0.61	0.79 ± 0.12	0.84	0.81	0.70	0.78 ± 0.04	0.79	1.40
1	1.23	0.92	0.96	1.04 ± 0.10	0.97	0.85	0.76	0.86 ± 0.06	0.95	1.79
2	1.21	0.79	0.85	0.95 ± 0.13	0.83	0.70	0.61	0.71 ± 0.06	0.96	1.20
3	1.26	0.85	0.93	1.01 ± 0.13	0.78	0.62	0.51	0.64 ± 0.08	0.62	1.17
4	1.41	0.86	1.04	1.10 ± 0.16	0.62	0.49	0.52	0.54 ± 0.04	0.30	1.12
5	1.36	0.87	0.99	1.07 ± 0.15	0.61	0.55	0.47	0.54 ± 0.04	0.34	0.97
6	1.10	0.84	0.75	0.90 ± 0.10	0.50	0.40	0.40	0.43 ± 0.03	0.25	0.68
7	1.04	0.73	0.83	0.87 ± 0.09	0.31	0.38	0.26	0.32 ± 0.03	0.27	0.74
8	1.11	0.86	0.91	0.96 ± 0.08	0.31	0.32	0.19	0.27 ± 0.04	0.14	0.68
9	1.28	0.79	1.08	1.05 ± 0.14	0.27	0.31	0.17	0.25 ± 0.04	0.16	0.55
10	1.26	0.81	1.04	1.04 ± 0.13	0.23	0.28	0.12	0.21 ± 0.05	0.18	0.59
11	0.92	0.67	0.94	0.84 ± 0.09	0.21	0.29	0.11	0.20 ± 0.05	0.11	0.66
12	0.82	0.63	0.74	0.73 ± 0.05	0.21	0.25	0.11	0.19 ± 0.04	0.18	0.62
13	1.03	0.63	0.84	0.83 ± 0.12	0.22	0.16	0.14	0.17 ± 0.02	0.10	0.69
14	0.81	0.55	0.62	0.66 ± 0.08	0.21	0.23	0.16	0.20 ± 0.02	0.13	0.63
15		0.66	0.80	0.73 ± 0.07		0.17	0.18	0.18 ± 0.00	0.18	0.60

+ Infected sheep without pair-fed control partners

Appendix 1: Table 18

Experiment 1: Serum Albumin:Globulin Ratios in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0	1.16	1.19	0.92	1.09 ± 0.09	0.98	1.05	0.82	0.95 ± 0.07	0.72	1.24
1	1.41	1.64	1.12	1.39 ± 0.15	1.29	1.05	0.94	1.09 ± 0.10	0.89	1.68
2	1.29	1.57	1.10	1.32 ± 0.14	0.95	1.03	0.89	0.96 ± 0.04	0.69	1.13
3	1.50	1.88	1.34	1.57 ± 0.16	0.77	1.05	0.89	0.90 ± 0.08	0.57	0.88
4	1.02	1.74	1.37	1.38 ± 0.21	0.52	0.96	0.77	0.75 ± 0.13	0.55	0.62
5	0.92	1.69	1.44	1.35 ± 0.23	0.54	0.95	0.74	0.74 ± 0.12	0.50	0.68
6	1.01	1.28	0.80	1.03 ± 0.14	0.45	0.77	0.44	0.55 ± 0.11	0.43	0.71
7	1.03	1.74	1.22	1.33 ± 0.21	0.50	0.81	0.41	0.57 ± 0.12	0.40	0.68
8	1.04	1.43	1.64	1.37 ± 0.18	0.58	0.65	0.56	0.60 ± 0.03	0.43	0.71
9	1.20	1.33	1.53	1.35 ± 0.10	0.43	0.45	0.46	0.45 ± 0.00	0.39	0.55
10	1.27	1.13	1.18	1.19 ± 0.04	0.31	0.51	0.29	0.37 ± 0.07	0.34	0.51
11	1.28	1.23	1.27	1.26 ± 0.01	0.24	0.49	0.25	0.33 ± 0.08	0.32	0.44
12	1.09	1.08	1.13	1.10 ± 0.14	0.21	0.42	0.25	0.29 ± 0.06	0.27	0.41
13	1.20	1.14	1.21	1.18 ± 0.02	0.21	0.34	0.21	0.25 ± 0.04	0.20	0.62
14	0.98	0.94	1.07	1.00 ± 0.04	0.18	0.40	0.16	0.25 ± 0.08	0.22	0.48
15	1.09	0.99	1.16	1.08 ± 0.05	0.30	0.49	0.24	0.34 ± 0.07	0.23	0.56
16	0.81	0.75	0.77	0.78 ± 0.02	0.25	0.26	0.20	0.24 ± 0.02	0.21	0.38
17	0.73	0.61	0.71	0.68 ± 0.04	0.26	0.17	0.21	0.21 ± 0.02	0.18	0.27
18	0.76	Dead	0.64	0.70 ± 0.06	0.21	Dead	0.17	0.19 ± 0.02	0.16	0.22
19	0.71		0.66	0.69 ± 0.02	0.24		0.12	0.18 ± 0.06	0.21	0.18
20	0.76		0.61	0.69 ± 0.07	0.19		0.14	0.17 ± 0.02	0.22	0.15

+ Infected sheep without pair-fed control partners

Appendix 1: Table 19

Experiment 1: Serum Glutamic Oxaloacetic Transaminase Concentration (I/u) in Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	985	992	Mean ± S.E.
0	24.0	50.0	14.8	29.6 ± 10.5	21.5	17.0	29.0	23.8	18.3	21.9 ± 2.1
1	20.5	44.0	16.0	26.8 ± 8.7	21.0	17.0	25.0	24.0	17.0	20.8 ± 1.7
2	22.5	32.0	19.5	24.7 ± 3.8	24.5	32.0	30.0	36.0	26.0	29.7 ± 2.1
3	27.5	30.5	19.5	25.8 ± 3.3	36.5	44.5	39.5	59.0	33.5	42.6 ± 4.5
4	26.3	30.3	18.8	25.1 ± 3.4	39.0	38.0	35.8	75.0	34.0	44.4 ± 7.7
5	24.0	22.5	18.5	21.7 ± 1.6	33.5	31.5	30.0	60.0	31.5	37.3 ± 5.7
6	21.0	28.0	21.0	23.3 ± 2.3	32.5	33.0	39.0	81.0	38.5	44.8 ± 9.1
7	25.0	21.5	20.0	22.2 ± 1.5	36.5	35.5	37.5	54.5	49.5	42.7 ± 3.9
8	24.5	19.5	18.5	20.8 ± 1.9	45.0	38.0	38.5	59.0	52.5	46.6 ± 4.1
9	20.0	19.5	18.5	19.3 ± 0.4	37.5	36.5	43.5	61.0	44.5	44.6 ± 4.4
10	25.0	21.5	23.5	23.3 ± 1.0	36.5	29.0	34.0	50.0	37.0	37.3 ± 3.5
11	25.0	18.0	19.5	20.8 ± 2.1	34.0	24.0	60.0	52.5	43.5	42.8 ± 6.4
12	26.0	13.0	15.5	18.2 ± 4.0	60.0	17.0	73.0	43.0	36.5	45.9 ± 9.7
13	42.0	17.5	21.0	26.8 ± 7.7	34.0	18.5	67.0	42.5	28.0	38.0 ± 8.2
14	55.0	14.5	16.5	28.7 ± 13.2	16.5	16.0	44.0	35.5	21.0	26.6 ± 5.6
15		22.0	18.5	20.3 ± 1.8		18.5	43.0	37.0	25.0	30.9 ± 5.6

Appendix 1: Table 20

Experiment 1: Serum Glutamic Oxaloacetic Transaminase Concentration (I/u) in Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	954	4	Mean ± S.E.
0	24.3	15.5	16.0	18.6 ± 2.9	24.8	24.3	20.0	21.8	17.8	21.7 ± 1.3
1	22.5	18.5	16.0	19.0 ± 1.9	27.5	24.0	20.0	19.5	19.5	22.1 ± 1.6
2	30.0	25.0	20.5	25.2 ± 2.7	37.0	31.5	28.5	35.0	30.0	32.4 ± 1.6
3	29.5	28.0	25.0	27.5 ± 1.3	56.0	41.5	38.5	60.5	53.0	49.9 ± 4.3
4	30.0	24.0	20.3	24.8 ± 2.8	50.8	46.3	42.5	45.3	52.3	47.4 ± 1.8
5	29.5	20.0	18.0	22.5 ± 3.5	31.0	43.0	38.0	36.0	41.0	37.8 ± 2.1
6	36.0	24.0	26.5	28.8 ± 3.7	52.5	49.5	40.0	44.5	42.0	45.7 ± 2.3
7	30.0	22.5	24.0	25.5 ± 2.3	41.0	55.0	66.0	54.0	36.0	50.4 ± 5.4
8	30.0	19.5	20.5	23.3 ± 3.3	38.0	53.5	41.0	45.0	46.0	44.7 ± 2.6
9	33.0	25.5	18.0	25.5 ± 4.3	42.5	51.0	41.5	46.0	44.5	45.1 ± 1.7
10	31.0	27.0	22.5	26.8 ± 2.5	41.0	34.0	35.0	43.0	36.5	37.9 ± 1.7
11	33.0	24.0	22.5	26.5 ± 3.3	46.5	32.5	27.5	37.5	29.0	34.6 ± 3.4
12	23.5	18.5	16.5	19.5 ± 2.1	75.0	24.0	21.5	26.5	21.5	33.7 ± 10.3
13	24.0	19.5	19.0	20.8 ± 1.6	82.5	24.5	23.5	31.5	23.0	37.0 ± 11.4
14	25.0	18.0	13.0	18.7 ± 3.5	97.5	20.5	19.5	28.5	16.5	36.5 ± 15.4
15	28.5	25.0	20.0	24.5 ± 2.5	100.0	23.5	27.5	33.0	21.0	41.0 ± 14.9
16	27.0	22.5	18.0	22.5 ± 2.6	78.0	25.0	33.5	31.5	21.0	37.8 ± 10.3
17	26.0	19.5	18.0	21.2 ± 2.5	95.5	38.5	32.0	40.0	20.0	45.2 ± 13.1
18	22.5	Dead	16.5	19.5 ± 3.0	62.0	Dead	25.0	29.0	18.5	33.6 ± 9.7
19	24.0		14.0	19.0 ± 5.0	47.0		22.5	24.0	16.0	27.4 ± 6.8
20	21.0		13.0	17.0 ± 4.0	43.5		33.0	33.0	20.0	32.4 ± 4.8

Appendix 1: Table 21

Experiment 1: Body Weights (kg) of Fluke-infected and Control Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985+	992+
-6	34.7	33.8	34.3	34.3 ± 0.3	32.8	31.3	35.8	33.3 ± 1.3	32.2	33.9
0	34.6	30.5	33.9	33.0 ± 1.3	34.3	29.7	33.0	32.3 ± 1.4	32.5	35.3
1	33.9	30.0	33.2	32.4 ± 1.2	34.1	29.6	32.0	31.9 ± 1.3	32.0	35.0
2	33.5	29.6	33.5	32.2 ± 1.3	33.9	29.8	31.5	31.7 ± 1.2	32.3	36.4
3	33.9	28.8	32.0	31.6 ± 1.5	34.2	30.4	31.0	31.9 ± 1.2	30.4	36.2
4	33.6	29.7	33.6	32.3 ± 1.3	33.3	29.2	31.0	31.2 ± 1.2	30.0	38.3
5	34.2	31.6	33.7	33.2 ± 0.8	33.1	29.0	30.6	30.9 ± 1.2	29.9	36.8
6	33.8	30.9	33.1	32.6 ± 0.9	32.9	29.7	31.1	31.2 ± 0.9	29.1	36.2
7	33.1	29.7	32.2	31.7 ± 1.0	31.5	28.6	30.2	30.1 ± 0.8	28.0	34.8
8	31.7	29.4	32.3	31.1 ± 0.9	30.8	27.7	28.9	29.1 ± 0.9	26.9	32.9
9	30.1	30.3	28.7	29.7 ± 0.5	27.9	27.8	27.1	27.6 ± 0.3	26.9	32.6
10	31.7	30.4	30.4	30.8 ± 0.4	28.8	27.7	27.8	28.1 ± 0.4	28.4	34.0
11	31.0	29.8	30.3	30.4 ± 0.3	29.3	27.3	28.6	28.4 ± 0.6	28.3	34.3
12	31.2	29.6	30.4	30.4 ± 0.5	29.4	27.7	28.8	28.6 ± 0.5	28.0	34.5
13	30.9	29.0	31.2	30.4 ± 0.7	29.3	27.0	27.9	28.1 ± 0.7	26.4	34.4
14	29.5	27.7	30.9	29.4 ± 0.9	27.5	26.4	27.4	27.1 ± 0.4	24.6	33.6
15	27.0	27.3	28.6	27.6 ± 0.5	26.3	26.6	27.1	26.7 ± 0.2	Dead	33.8

+ Infected sheep without pair-fed control partners

Appendix 1: Table 22

Experiment 1: Body weights (kg) of Fluke-infected and Control Sheep Fed Hay and Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
-6	33.4	34.6	36.2	34.7 ± 0.8	33.5	32.9	33.1	33.2 ± 0.2	32.1	33.9
0	37.9	35.2	36.2	36.4 ± 0.8	37.4	36.8	35.8	36.7 ± 0.5	34.3	36.3
1	37.0	35.5	36.4	36.3 ± 0.4	37.5	36.8	35.5	36.6 ± 0.6	33.9	36.1
2	36.7	35.9	36.1	36.2 ± 0.2	37.4	37.1	35.6	36.7 ± 0.6	33.5	35.7
3	37.2	36.4	36.5	36.7 ± 0.3	38.1	37.2	36.4	37.2 ± 0.5	32.7	35.6
4	37.3	37.0	36.7	37.0 ± 0.2	36.6	37.6	36.8	37.0 ± 0.3	32.9	35.2
5	35.8	36.8	36.9	36.5 ± 0.4	36.3	37.8	36.3	36.8 ± 0.5	33.2	35.6
6	35.4	36.2	36.2	35.9 ± 0.3	35.9	37.9	35.2	36.3 ± 0.8	32.0	36.2
7	35.2	35.9	35.0	35.4 ± 0.3	35.2	37.3	34.7	35.7 ± 0.8	31.4	34.8
8	36.0	35.4	34.9	35.4 ± 0.3	34.7	36.2	34.1	35.0 ± 0.6	31.7	34.7
9	35.2	34.6	34.7	34.8 ± 0.2	34.1	35.7	33.3	34.4 ± 0.7	31.5	34.3
10	35.3	35.0	35.0	35.1 ± 0.1	34.2	35.7	32.9	34.3 ± 0.8	31.3	34.6
11	35.4	35.5	34.6	35.2 ± 0.3	33.1	36.8	32.6	34.2 ± 1.3	30.5	35.3
12	36.4	36.3	35.5	36.1 ± 0.3	33.7	37.3	32.8	34.6 ± 1.4	31.1	35.9
13	37.1	35.6	35.2	36.0 ± 0.6	33.8	37.4	31.8	34.3 ± 1.6	30.4	36.2
14	36.5	35.4	34.9	35.6 ± 0.5	33.9	36.7	31.5	34.0 ± 1.5	30.5	36.2
15	36.8	34.7	34.4	35.3 ± 0.8	33.9	37.9	30.1	34.0 ± 2.3	30.4	35.8
16	36.3	34.0	35.5	35.3 ± 0.7	33.0	38.1	31.2	34.1 ± 2.1	30.9	34.9
17	36.1	31.7	35.7	34.5 ± 1.4	33.4	37.5	31.3	34.1 ± 1.8	31.0	34.9
18	36.5	Dead	34.7	35.6 ± 0.9	32.7	Dead	29.6	31.2 ± 1.6	30.0	35.4
19	36.4		33.5	35.0 ± 1.5	32.7		30.1	31.4 ± 1.3	30.4	32.6
20	35.0		32.3	33.7 ± 1.4	31.3		30.3	30.8 ± 0.5	30.2	31.6

+ Infected sheep without pair-fed control partners

Appendix 1: Table 23

Experiment 1: Hay Intake (g dry matter/day) of Fluke-infected and Control Sheep Fed Hay Only

Days After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
0 - 6	895	772	823	830 ± 36	806	686	857	783 ± 51	903	1067
6 - 11	729	622	758	703 ± 41	811	670	754	745 ± 41	766	888
11 - 16	764	662	695	707 ± 30	788	675	729	731 ± 33	821	935
16 - 22	843	566	622	677 ± 85	870	699	702	757 ± 57	642	944
22 - 28	747	628	720	698 ± 36	702	614	729	682 ± 35	679	1099
28 - 33	622	741	846	736 ± 65	599	760	867	742 ± 78	654	966
33 - 38	865	726	808	800 ± 40	840	896	805	847 ± 27	757	1027
38 - 43	796	789	803	796 ± 4	802	794	821	806 ± 8	789	1046
43 - 48	827	746	775	783 ± 24	837	754	786	792 ± 24	742	980
48 - 55	680	659	727	689 ± 20	658	655	671	661 ± 5	698	853
55 - 60	444	557	371	457 ± 54	447	595	359	467 ± 69	380	599
60 - 65	467	648	488	534 ± 57	488	654	496	546 ± 54	577	767
65 - 69	718	738	651	702 ± 26	714	705	675	698 ± 12	716	905
69 - 75	704	675	689	689 ± 8	698	673	721	697 ± 14	773	935
75 - 81	672	612	687	657 ± 23	658	615	639	637 ± 12	827	921
81 - 86	678	646	723	683 ± 22	672	632	707	670 ± 22	764	948
86 - 91	647	578	700	642 ± 35	636	560	687	628 ± 37	717	900
91 - 96	523	532	713	589 ± 62	513	528	734	592 ± 71	723	677
96 - 103	310	482	563	452 ± 75	295	478	586	453 ± 85	598	832
103 - 106		380	664	522 ± 142		313	686	500 ± 187		800

* Infected sheep without pair-fed control partners.

Appendix 1: Table 24

Experiment 1: Hay Intake (g dry matter/day) of Fluke-infected and Control Sheep fed Hay and Compound Diet

Days After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
0 - 6	690	622	601	638 ± 27	628	567	583	593 ± 18	614	589
6 - 11	593	622	474	563 ± 45	608	570	500	559 ± 32	447	438
11 - 16	523	653	518	565 ± 44	509	649	553	570 ± 41	419	411
16 - 22	525	555	552	544 ± 9	522	571	534	542 ± 15	408	434
22 - 28	459	617	533	536 ± 46	429	625	548	534 ± 57	325	319
28 - 33	462	745	675	627 ± 85	503	734	645	627 ± 67	457	373
33 - 38	557	725	574	619 ± 53	579	740	590	636 ± 52	540	441
38 - 43	547	824	506	626 ± 100	567	825	503	632 ± 98	562	561
43 - 48	536	748	486	590 ± 80	543	733	501	592 ± 71	466	490
48 - 55	445	539	348	444 ± 55	416	518	342	425 ± 51	390	491
55 - 60	290	371	257	306 ± 34	279	384	280	314 ± 35	270	271
60 - 65	341	434	337	371 ± 32	335	442	328	368 ± 37	305	418
65 - 69	393	565	390	449 ± 58	382	599	388	456 ± 71	394	446
69 - 75	351	553	259	388 ± 87	340	509	252	367 ± 75	402	471
75 - 81	454	607	364	475 ± 71	358	548	333	413 ± 68	464	536
81 - 86	546	583	582	570 ± 12	536	583	560	560 ± 14	544	632
86 - 91	535	556	569	553 ± 10	503	560	580	548 ± 23	592	618
91 - 96	508	489	409	469 ± 30	562	507	441	503 ± 35	463	559
96 - 103	497	466	312	425 ± 57	508	459	307	425 ± 61	380	511
103 - 110	451	366	327	381 ± 37	426	281	355	354 ± 42	379	393
110 - 115	539	144	421	368 ± 117	512	133	416	354 ± 114	466	406
115 - 122	446	117	299	287 ± 95	419	99	271	263 ± 92	428	360
122 - 127	481	Dead	230	356 ± 126	447	Dead	230	339 ± 109	402	277
127 - 132	487		222	355 ± 133	437		222	330 ± 108	370	182
132 - 137	460		239	350 ± 111	444		179	312 ± 133	433	80

* Infected sheep without pair-fed control partners

Appendix 1: Table 25

Experiment 1: Compound Diet Intake (g dry matter/day) of Fluke-infected and Control Sheep Fed Hay and Compound Diet

Days After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954+	4+
0 - 69	476	476	476	476 ± 0	476	476	476	476 ± 0	476	476
69 - 75	476	476	476	476 ± 0	476	476	476	476 ± 0	476	276
75 - 81	399	476	209	361 ± 79	399	476	188	354 ± 86	285	340
81 - 86	476	476	238	397 ± 79	476	476	238	397 ± 79	209	476
86 - 91	476	476	323	425 ± 51	476	476	361	438 ± 38	399	476
91 - 110	476	476	476	476 ± 0	476	476	476	476 ± 0	476	476
110 - 115	476	437	476	463 ± 13	476	437	476	463 ± 13	476	476
115 - 122	476	136	476	363 ± 113	476	136	476	363 ± 113	476	476
122 - 127	476	Dead	476	476 ± 0	476	Dead	476	476 ± 0	476	476
127 - 132	476		437	457 ± 20	476		437	457 ± 20	476	476
132 - 137	476		380	428 ± 48	476		380	428 ± 48	476	399

+ Infected sheep without pair-fed control partners

Appendix 1: Table 26

Experiment 1: Crude Protein Intake (g/day) of Fluke-infected and Control Sheep Fed Hay Only

Days After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
0 - 6	48.8	42.2	44.9	45.3 ± 1.9	44.0	37.5	46.8	42.8 ± 2.8	51.4	58.2
6 - 11	47.9	40.9	49.9	46.2 ± 2.7	53.4	44.1	49.6	49.0 ± 2.7	50.4	58.4
11 - 16	63.8	55.3	58.1	59.1 ± 2.5	65.8	56.4	60.9	61.0 ± 2.7	68.6	78.1
16 - 22	70.5	47.3	52.0	56.6 ± 7.1	72.6	58.4	58.7	63.2 ± 4.7	53.7	79.0
22 - 28	70.7	59.5	68.2	66.1 ± 3.4	66.5	58.1	69.0	64.5 ± 3.3	64.4	104.1
28 - 33	25.8	30.7	35.1	30.5 ± 2.7	24.8	31.5	35.9	30.8 ± 3.2	27.1	41.3
33 - 38	45.5	38.2	42.5	42.1 ± 2.1	44.2	47.2	42.4	44.6 ± 1.4	39.9	54.1
38 - 43	52.9	52.4	53.3	52.9 ± 0.3	53.3	52.8	54.5	53.5 ± 0.5	52.5	69.5
43 - 48	54.9	49.6	51.5	52.0 ± 1.6	55.6	50.1	52.2	52.6 ± 1.6	49.3	65.1
48 - 55	45.1	43.8	48.3	45.7 ± 1.3	43.7	43.5	44.6	43.9 ± 0.3	46.3	56.7
55 - 60	43.2	54.2	36.1	44.5 ± 5.3	43.6	57.9	35.0	45.5 ± 6.7	37.0	58.3
60 - 65	45.4	63.1	47.5	52.0 ± 5.6	47.5	63.6	48.3	53.1 ± 5.2	56.1	74.6
65 - 69	39.2	40.3	35.5	38.3 ± 1.5	39.0	38.5	36.8	38.1 ± 0.7	39.1	49.4
69 - 75	36.6	35.1	35.8	35.8 ± 0.4	36.3	33.9	37.5	35.9 ± 1.1	40.2	48.6
75 - 81	34.9	31.8	35.7	34.1 ± 1.2	34.2	31.9	33.2	33.1 ± 0.7	43.0	47.8
81 - 86	33.5	32.0	35.7	33.7 ± 1.1	33.1	31.2	34.9	33.1 ± 1.1	37.7	46.8
86 - 91	31.9	28.5	34.5	31.6 ± 1.7	31.4	27.6	33.9	31.0 ± 1.8	35.4	44.4
91 - 96	25.8	26.3	35.2	29.1 ± 3.1	25.3	26.1	36.2	29.2 ± 3.5	35.7	33.4
96 - 103	14.2	22.1	25.8	20.7 ± 3.4	13.5	21.9	26.9	20.8 ± 3.9	27.4	38.2
103 - 106		18.8	32.8	25.8 ± 7.0		15.4	33.8	24.6 ± 9.2		39.5

* Infected sheep without pair-fed control partners.

Appendix 1: Table 27

Experiment 1: Crude Protein Intake (g/day) of Fluke-infected and Control Sheep Fed Hay and Compound Diet

Days After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
0 - 6	111.3	107.6	106.4	108.4 ± 1.5	107.9	104.6	105.5	106.0 ± 1.0	104.8	103.5
6 - 11	110.3	112.2	102.5	108.3 ± 3.0	111.3	108.8	104.3	108.1 ± 2.1	100.7	100.1
11 - 16	115.0	125.8	114.6	118.5 ± 3.7	113.8	125.5	117.5	118.9 ± 3.5	106.3	105.7
16 - 22	118.8	121.2	121.0	120.3 ± 0.8	118.5	122.6	119.6	120,2 ± 1.2	105.4	107.6
22 - 28	98.5	107.8	102.9	103.1 ± 2.7	96.7	108.3	103.8	102.9 ± 3.4	90.5	90.2
28 - 33	90.4	102.2	99.3	97.3 ± 3.6	92.1	101.7	98.0	97.3 ± 2.8	90.2	86.8
33 - 38	90.3	109.1	101.2	100.2 ± 5.5	101.4	109.9	102.0	104.4 ± 2.7	99.7	94.5
38 - 43	107.2	125.6	104.6	112.5 ± 6.6	108.6	125.6	104.4	112.9 ± 6.5	108.7	108.6
43 - 48	106.5	120.5	103.2	110.1 ± 5.3	107.0	119.5	104.3	110.3 ± 4.7	102.3	103.8
48 - 55	102.2	109.6	96.8	102.9 ± 3.7	101.3	108.2	96.4	102.0 ± 3.4	97.2	103.9
55 - 60	99.1	105.1	96.0	100.1 ± 2.7	97.9	108.1	98.2	101.4 ± 3.4	97.7	97.7
60 - 65	104.0	113.0	103.7	106.9 ± 3.1	103.4	113.7	102.7	106.6 ± 3.6	101.0	112.0
65 - 69	92.5	101.8	92.4	95.6 ± 3.1	91.9	103.6	92.2	95.9 ± 3.9	92.8	95.6
69 - 75	91.8	102.3	87.1	93.7 ± 4.5	90.1	102.7	86.6	93.1 ± 4.9	92.2	86.0
75 - 81	73.8	103.6	75.9	84.4 ± 9.6	72.3	102.7	76.8	83.9 ± 9.5	67.0	78.9
81 - 86	86.7	99.8	58.3	81.6 ± 12.2	86.2	99.8	58.3	81.4 ± 12.2	58.5	102.5
86 - 91	97.4	98.4	56.0	83.9 ± 14.0	95.9	98.7	56.5	83.7 ± 13.6	89.2	101.8
91 - 96	97.7	95.2	75.8	89.6 ± 6.9	97.2	96.1	75.6	89.6 ± 7.0	94.1	98.9
96 - 103	97.6	96.1	87.0	93.6 ± 3.3	89.2	95.7	86.1	93.3 ± 3.7	90.1	96.5
103 - 110	93.3	89.2	87.2	89.9 ± 1.8	94.2	87.4	89.8	90.5 ± 2.0	89.8	90.4
110 - 115	97.4	80.1	91.7	89.7 ± 5.1	96.0	78.9	91.4	88.8 ± 5.1	94.0	91.0
115 - 122	94.7	27.5	88.0	70.1 ± 21.4	93.5	26.0	86.3	68.6 ± 21.4	92.1	88.8
122 - 127	106.1	Dead	88.0	97.1 ± 9.1	103.6	Dead	87.9	95.8 ± 7.9	100.6	91.6
127 - 132	106.5		81.6	94.1 ± 12.5	102.9		81.5	92.2 ± 10.7	98.3	84.6
132 - 137	104.6		65.9	85.3 ± 19.4	103.4		64.3	83.9 ± 19.6	102.9	65.8

* Infected sheep without pair-fed control partners.

Appendix 1: Table 28

Experiment 2: Adult Flukes recovered from sheep 20 weeks after infection with 600 *F. hepatica* metacercariae

Sheep No.	Number of Flukes
78	120
77	121
60	123
68	419
54	231
61	182
Mean	199
S.E.	47

Appendix 1: Table 29

Experiment 2: Packed cell volumes (%) in sheep following infection with *F. hepatica* and worm-free controls

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-1	29.5	31.5	31.0	32.0	33.0	32.5	31.6 ± 0.5	34.0	30.0	30.0	33.0	30.5	31.0	31.4 ± 0.7
0	31.0	31.0	33.0	34.0	33.5	32.0	32.4 ± 0.5	33.0	29.0	34.0	35.0	31.0	32.0	32.3 ± 0.9
1	36.0	32.5	35.0	33.5	37.0	32.0	34.3 ± 0.8	34.5	33.5	34.0	35.5	32.5	34.0	34.0 ± 0.4
2	30.0	31.0	31.0	34.0	31.0	30.0	31.2 ± 0.6	32.5	30.0	33.0	34.0	31.0	29.0	31.6 ± 0.8
3	31.0	31.5	31.0	32.5	32.0	32.0	31.7 ± 0.2	33.0	29.5	33.0	33.5	30.0	30.0	31.5 ± 0.8
4	29.0	29.0	31.5	29.0	32.5	30.0	30.2 ± 0.6	30.5	29.0	32.0	33.0	31.0	31.0	31.1 ± 0.6
5	30.5	29.0	30.0	29.5	30.0	30.5	29.9 ± 0.2	31.0	28.0	31.5	30.5	30.0	29.0	30.0 ± 0.5
6	30.0	31.5	30.5	32.5	31.0	32.0	31.3 ± 0.4	30.0	28.0	29.0	29.0	29.5	28.5	29.0 ± 0.3
7	29.0	29.0	30.5	32.0	31.0	31.5	30.5 ± 0.5	26.0	26.5	28.0	26.5	29.0	26.5	27.1 ± 0.5
8	26.5	27.0	28.0	30.0	31.0	29.0	28.6 ± 0.7	27.0	25.0	26.5	23.0	26.0	28.0	25.9 ± 0.7
9	28.0	28.0	29.0	32.5	29.0	30.0	29.4 ± 0.7	25.0	23.5	24.0	19.0	25.0	25.0	23.6 ± 1.0
10	31.5	30.0	29.0	29.0	29.5	30.0	29.8 ± 0.4	25.5	22.0	24.0	21.5	26.0	25.0	24.0 ± 0.8
11	30.5	29.0	28.0	31.0	30.0	31.0	29.9 ± 0.5	23.5	24.0	24.0	22.0	24.0	23.5	23.5 ± 0.3
12	27.0	26.5	29.0	29.5	29.0	29.0	28.3 ± 0.5	22.0	24.0	24.0	21.0	24.0	24.5	23.3 ± 0.6
13	30.0	29.5	28.0	29.0	27.0	27.5	28.5 ± 0.5	22.0	22.0	22.0	20.5	24.0	23.5	22.3 ± 0.5
14	28.0	28.5	29.0	31.0	29.0	32.0	29.6 ± 0.6	23.5	25.5	23.0	22.0	24.5	25.0	23.9 ± 0.5
15	29.5	29.5	26.0	31.0	30.0	30.0	29.3 ± 0.7	23.0	23.0	21.5	20.0	22.0	21.0	21.8 ± 0.5
16	27.5	27.5	27.0	30.0	32.0	30.0	29.0 ± 0.8	25.0	22.0	21.0	21.0	22.5	22.0	22.3 ± 0.6
17	31.0	29.5	30.0	35.0	30.5	29.5	30.9 ± 0.9	21.0	22.0	18.0	19.0	21.0	21.0	20.3 ± 0.6
18	30.0	30.0	28.5	35.0	31.5	33.0	31.3 ± 1.0	20.0	20.0	15.0	17.0	20.0	20.0	18.7 ± 0.9
19	28.5	30.5	29.0	33.0	30.0	30.5	30.3 ± 0.6	19.0	20.0	16.0	16.0	20.0	19.0	18.3 ± 0.8
20	29.0	28.5	26.5	33.0	30.0	31.0	29.7 ± 0.9	20.5	19.0	15.5	12.0	18.0	19.0	17.3 ± 1.3

Appendix 1: Table 30

Experiment 2: Total serum protein concentrations (g/100ml) in sheep following infection with *F. hepatica* and worm-free controls

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-1	5.48	6.23	5.96	6.18	5.93	5.68	5.91 ± 0.12	5.72	5.95	5.96	5.86	5.58	5.87	5.82 ± 0.06
0	5.38	6.10	5.78	6.41	5.94	5.55	5.86 ± 0.15	5.78	5.85	5.90	5.89	5.55	5.60	5.76 ± 0.06
1	5.57	6.03	5.76	6.25	5.80	5.62	5.84 ± 0.11	5.71	5.78	5.93	5.65	5.75	5.69	5.75 ± 0.04
2	5.27	6.33	5.60	6.22	5.75	5.56	5.80 ± 0.17	5.88	5.85	6.05	5.65	5.59	5.75	5.80 ± 0.07
3	5.27	6.15	5.62	6.04	5.71	5.46	5.71 ± 0.14	5.87	5.77	6.12	5.60	5.59	5.76	5.79 ± 0.08
4	5.30	5.93	5.63	6.00	5.82	5.63	5.71 ± 0.10	6.25	5.98	6.18	5.84	5.54	5.92	5.95 ± 0.10
5	5.50	5.85	5.50	5.67	5.83	5.45	5.63 ± 0.07	6.30	5.76	6.02	5.75	5.74	5.88	5.91 ± 0.09
6	5.22	5.88	5.55	5.69	5.84	5.70	5.65 ± 0.10	6.78	5.92	6.20	6.03	5.78	5.97	6.11 ± 0.14
7	5.20	5.85	5.74	5.83	5.75	5.40	5.63 ± 0.11	6.90	6.13	6.38	6.46	6.02	6.09	6.33 ± 0.13
8	5.35	5.97	5.72	6.14	5.70	5.55	5.73 ± 0.12	7.02	6.43	6.28	6.37	6.15	6.20	6.41 ± 0.13
9	5.15	5.72	5.51	5.93	5.75	5.59	5.61 ± 0.11	6.93	6.37	6.15	6.40	6.48	6.35	6.45 ± 0.11
10	5.47	6.00	5.71	5.85	5.62	5.46	5.69 ± 0.09	7.15	6.42	6.02	6.52	6.48	6.28	6.48 ± 0.15
11	5.61	5.92	5.62	5.92	5.52	5.35	5.66 ± 0.09	7.52	6.32	5.82	6.40	6.33	6.20	6.43 ± 0.24
12	5.70	5.94	5.70	5.78	5.80	5.43	5.73 ± 0.07	7.40	6.38	5.82	5.87	5.97	5.96	6.23 ± 0.25
13	5.55	5.75	5.64	6.01	5.96	5.52	5.74 ± 0.08	7.50	6.10	6.06	5.39	5.84	6.00	6.14 ± 0.29
14	5.72	5.80	5.55	5.89	5.91	5.52	5.73 ± 0.07	7.52	6.06	5.84	5.42	5.91	5.67	6.07 ± 0.30
15	5.77	6.00	5.65	5.90	5.91	5.69	5.82 ± 0.06	7.58	6.12	5.80	5.30	5.92	5.83	6.09 ± 0.32
16	5,78	5.95	5.73	5.82	5.85	5.67	5.80 ± 0.04	7.48	6.07	5.79	5.30	5.80	5.53	6.00 ± 0.32
17	5.85	5.95	5.66	6.19	5.91	5.58	5.86 ± 0.09	7.32	5.90	5.80	4.80	5.62	5.60	5.84 ± 0.34
18	5.63	5.82	5.52	6.10	5.74	5.60	5.74 ± 0.08	6.98	5.66	5.78	4.23	5.16	5.24	5.51 ± 0.37
19	5.72	5.76	5.74	5.81	5.96	5.68	5.78 ± 0.04	6.90	5.45	5.04	4.00	4.82	5.25	5.24 ± 0.39
20	5.47	6.03	5.72	6.11	5.85	5.46	5.77 ± 0.11	6.85	5.32	4.45	3.53	4.49	5.32	4.99 ± 0.46

Appendix 1: Table 31

Experiment 2: Serum albumin concentrations (g/100ml) in sheep following infection with F. hepatica and worm-free controls

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-1	3.40	3.69	3.35	3.35	3.62	3.35	3.46 ± 0.06	3.52	3.63	3.20	3.50	3.54	3.50	3.48 ± 0.06
0	3.65	3.65	3.52	3.38	3.63	3.44	3.55 ± 0.05	3.75	3.81	3.12	3.33	3.59	3.72	3.55 ± 0.11
1	3.66	3.65	3.46	3.43	3.60	3.60	3.57 ± 0.04	3.78	3.60	3.06	3.30	3.64	3.65	3.51 ± 0.11
2	3.46	3.92	3.37	3.35	3.66	3.61	3.56 ± 0.09	3.55	3.72	3.02	3.41	3.50	3.67	3.48 ± 0.10
3	3.42	3.95	3.50	3.24	3.72	3.60	3.57 ± 0.10	3.49	3.88	3.25	3.44	3.69	3.53	3.55 ± 0.09
4	3.55	3.90	3.38	3.06	3.67	3.70	3.54 ± 0.12	3.32	3.75	3.22	3.30	3.33	3.33	3.38 ± 0.08
5	3.61	3.68	3.35	2.83	3.85	3.44	3.46 ± 0.15	3.11	3.72	3.03	3.16	3.34	3.35	3.29 ± 0.10
6	3.35	3.59	3.64	3.05	3.53	3.39	3.42 ± 0.08	3.02	3.73	3.05	3.09	3.02	3.32	3.21 ± 0.11
7	3.41	3.55	3.58	3.06	3.51	3.38	3.42 ± 0.08	3.04	3.60	3.10	3.17	2.98	3.35	3.21 ± 0.09
8	3.30	3.62	3.50	3.09	3.61	3.30	3.40 ± 0.09	2.97	3.45	2.87	3.02	2.65	3.28	3.04 ± 0.12
9	3.42	3.64	3.49	3.05	3.64	3.29	3.42 ± 0.09	2.90	3.45	2.95	2.76	2.46	3.33	2.98 ± 0.15
10	3.51	3.72	3.57	3.02	3.74	3.35	3.49 ± 0.11	3.01	3.47	2.67	2.49	2.45	3.25	2.89 ± 0.17
11	3.47	3.70	3.50	3.07	3.82	3.34	3.48 ± 0.11	2.87	3.57	2.62	2.40	2.52	3.00	2.83 ± 0.17
12	3.51	3.52	3.42	3.15	3.65	3.42	3.45 ± 0.07	2.70	3.47	2.67	2.23	2.46	2.94	2.74 ± 0.18
13	3.47	3.58	3.42	2.96	3.72	3.45	3.43 ± 0.10	2.68	3.58	2.63	2.23	2.53	2.70	2.73 ± 0.19
14	3.65	3.58	3.42	3.05	3.83	3.45	3.49 ± 0.11	2.67	3.47	2.75	2.23	2.64	2.72	2.75 ± 0.16
15	3.60	3.62	3.28	2.96	3.72	3.43	3.44 ± 0.11	2.65	3.45	2.70	1.79	2.33	2.32	2.54 ± 0.23
16	3.47	3.65	3.33	3.00	3.70	3.52	3.45 ± 0.10	2.59	3.42	2.68	1.50	2.19	2.24	2.44 ± 0.26
17	3.41	3.50	3.42	2.88	3.63	3.35	3.37 ± 0.10	2.32	2.96	2.65	1.38	2.18	2.50	2.33 ± 0.22
18	3.46	3.44	3.43	3.08	3.58	3.37	3.39 ± 0.07	2.10	2.85	2.70	1.20	2.06	2.08	2.17 ± 0.24
19	3.40	3.22	3.40	3.15	3.58	3.32	3.34 ± 0.06	2.10	2.51	2.15	1.00	1.83	2.02	1.94 ± 0.21
20	3.27	3.25	3.32	2.94	3.63	3.11	3.25 ± 0.09	1.93	2.32	2.17	0.77	1.23	1.94	1.73 ± 0.24

Appendix 1: Table 32

Experiment 2: Serum globulin concentrations (g/100ml) in sheep following infection with *F. hepatica* and worm-free controls

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-1	2.08	2.54	2.61	2.83	2.31	2.33	2.45 ± 0.11	2.20	2.32	2.76	2.36	2.04	2.37	2.34 ± 0.10
0	1.73	2.45	2.26	3.03	2.31	2.11	2.32 ± 0.17	2.03	2.04	2.78	2.56	1.96	1.88	2.21 ± 0.15
1	1.91	2.38	2.30	2.82	2.20	2.02	2.27 ± 0.13	1.93	2.18	2.87	2.35	2.11	2.04	2.25 ± 0.14
2	1.81	2.41	2.23	2.87	2.09	1.95	2.23 ± 0.15	2.33	2.13	3.03	2.24	2.09	2.08	2.32 ± 0.15
3	1.85	2.20	2.12	2.80	1.99	1.86	2.13 ± 0.14	2.38	1.89	2.87	2.16	1.90	2.23	2.24 ± 0.14
4	1.75	2.03	2.25	2.94	2.15	1.63	2.13 ± 0.19	2.93	2.23	2.96	2.54	2.21	2.59	2.58 ± 0.13
5	1.89	2.17	2.15	2.84	1.98	2.01	2.17 ± 0.14	3.19	2.04	2.99	2.59	2.40	2.53	2.61 ± 0.16
6	1.87	2.29	1.91	2.64	2.31	2.31	2.22 ± 0.12	3.76	2.19	3.15	2.94	2.76	2.65	2.91 ± 0.22
7	1.89	2.30	2.16	2.77	2.24	2.02	2.23 ± 0.12	3.86	2.53	3.28	3.29	3.04	2.74	3.12 ± 0.19
8	2.05	2.35	2.22	3.05	2.09	2.25	2.34 ± 0.15	4.05	2.98	3.41	3.35	3.40	2.92	3.35 ± 0.17
9	1.73	2.08	2.02	2.88	2.11	2.30	2.19 ± 0.16	4.03	2.92	3.20	3.64	4.02	3.02	3.47 ± 0.21
10	1.96	2.28	2.14	2.83	1.88	2.11	2.20 ± 0.14	4.14	2.95	3.35	4.03	4.03	3.03	3.59 ± 0.22
11	2.14	2.22	2.12	2.85	1.70	2.01	2.17 ± 0.15	4.65	2.75	3.20	4.00	3.81	3.20	3.60 ± 0.28
12	2.19	2.42	2.28	2.63	2.15	2.01	2.28 ± 0.09	4.70	2.91	3.15	3.64	3.51	3.02	3.49 ± 0.27
13	2.08	2.17	2.22	3.05	2.24	2.07	2.31 ± 0.15	4.82	2.52	3.43	3.16	3.31	3.30	3.42 ± 0.31
14	2.07	2.22	2.12	2.84	2.08	2.07	2.23 ± 0.12	4.85	2.59	3.09	3.19	3.27	2.95	3.32 ± 0.32
15	2.17	2.38	2.37	2.94	2.19	2.26	2.39 ± 0.11	4.93	2.66	3.10	3.51	3.59	3.31	3.52 ± 0.31
16	2.31	2.30	2.40	2.82	2.15	2.15	2.36 ± 0.10	4.89	2.65	3.11	3.80	3.61	3.29	3.55 ± 0.31
17	2.44	2.45	2.24	3.31	2.28	2.23	2.49 ± 0.17	5.00	2.94	3.15	3.42	3.44	3.10	3.51 ± 0.31
18	2.17	2.38	2.09	3.02	2.16	2.23	2.34 ± 0.14	4.88	2.81	3.08	3.03	3.10	3.16	3.34 ± 0.31
19	2.32	2.54	2.34	2.66	2.38	2.36	2.43 ± 0.06	4.80	2.94	2.88	3.00	2.99	3.23	3.30 ± 0.30
20	2.20	2.78	2.40	3.17	2.22	2.35	2.52 ± 0.16	4.92	3.00	2.28	2.76	3.26	3.38	3.27 ± 0.37

Appendix 1: Table 33

Experiment 2: Serum albumin:globulin ratios in sheep following infection with *F. hepatica* and worm-free controls

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-1	1.63	1.45	1.28	1.18	1.57	1.43	1.42 ± 0.07	1.60	1.56	1.16	1.48	1.73	1.47	1.50 ± 0.08
0	2.09	1.49	1.56	1.12	1.57	1.63	1.58 ± 0.13	1.84	1.87	1.12	1.30	1.83	1.98	1.66 ± 0.14
1	1.92	1.54	1.50	1.22	1.63	1.78	1.60 ± 0.10	1.96	1.65	1.06	1.40	1.73	1.79	1.60 ± 0.13
2	1.91	1.63	1.51	1.16	1.75	1.85	1.64 ± 0.11	1.52	1.75	1.00	1.52	1.67	1.76	1.54 ± 0.12
3	1.85	1.79	1.65	1.16	1.86	1.93	1.71 ± 0.12	1.46	2.05	1.13	1.59	1.94	1.58	1.63 ± 0.14
4	2.02	1.92	1.50	1.04	1.71	2.27	1.74 ± 0.18	1.13	1.68	1.09	1.29	1.51	1.28	1.33 ± 0.09
5	1.91	1.69	1.56	1.00	1.94	1.71	1.64 ± 0.14	0.97	1.82	1.01	1.22	1.39	1.32	1.29 ± 0.13
6	1.79	1.57	1.91	1.16	1.53	1.47	1.57 ± 0.11	0.80	1.70	0.97	1.05	1.09	1.25	1.14 ± 0.13
7	1.80	1.54	1.66	1.10	1.57	1.67	1.56 ± 0.10	0.78	1.42	0.95	0.96	0.98	1.22	1.05 ± 0.09
8	1.61	1.54	1.58	1.01	1.72	1.47	1.49 ± 0.10	0.73	1.16	0.84	0.90	0.78	1.12	0.92 ± 0.07
9	1.98	1.75	1.73	1.06	1.73	1.43	1.61 ± 0.13	0.72	1.18	0.92	0.76	0.61	1.10	0.88 ± 0.09
10	1.79	1.63	1.67	1.07	1.99	1.59	1.62 ± 0.14	0.73	1.18	0.80	0.62	0.61	1.07	0.84 ± 0.10
11	1.62	1.67	1.65	1.08	2.25	1.66	1.66 ± 0.15	0.62	1.30	0.82	0.60	0.66	0.94	0.82 ± 0.11
12	1.60	1.45	1.50	1.20	1.70	1.70	1.53 ± 0.08	0.57	1.19	0.85	0.61	0.70	0.97	0.82 ± 0.10
13	1.67	1.65	1.54	0.97	1.66	1.67	1.53 ± 0.11	0.56	1.42	0.77	0.71	0.76	0.82	0.84 ± 0.12
14	1.76	1.61	1.62	1.07	1.84	1.67	1.60 ± 0.11	0.55	1.34	0.89	0.70	0.81	0.92	0.87 ± 0.11
15	1.66	1.52	1.38	1.01	1.70	1.52	1.47 ± 0.10	0.54	1.30	0.87	0.51	0.65	0.70	0.76 ± 0.12
16	1.56	1.59	1.37	1.06	1.72	1.64	1.49 ± 0.10	0.53	1.29	0.86	0.39	0.61	0.68	0.73 ± 0.13
17	1.40	1.43	1.53	0.87	1.59	1.50	1.39 ± 0.11	0.46	1.01	0.84	0.40	0.63	0.81	0.69 ± 0.10
18	1.59	1.45	1.64	1.04	1.66	1.49	1.48 ± 0.09	0.43	1.01	0.88	0.40	0.66	0.66	0.67 ± 0.10
19	1.47	1.27	1.45	1.18	1.50	1.41	1.38 ± 0.05	0.44	0.85	0.75	0.33	0.61	0.63	0.60 ± 0.08
20	1.49	1.17	1.38	0.93	1.64	1.32	1.32 ± 0.10	0.39	0.77	0.95	0.28	0.38	0.57	0.56 ± 0.11

Appendix 1: Table 34

Experiment 2: Body Weight (kg) changes in sheep following infection with F. hepatica and worm-free controls

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-1	25.2	26.5	22.4	23.6	25.7	27.7	25.2 ± 0.8	26.5	28.6	21.1	22.1	25.6	27.7	25.3 ± 1.2
0	26.5	27.7	22.9	25.1	26.8	28.9	26.3 ± 0.9	27.3	29.0	22.1	22.6	26.5	28.9	26.1 ± 1.2
1	26.2	28.8	23.5	25.4	27.7	29.6	26.9 ± 0.9	27.4	31.2	22.7	22.8	27.8	29.6	26.9 ± 1.4
2	27.3	29.5	24.6	26.3	28.6	30.5	27.8 ± 0.9	27.9	31.6	22.9	23.1	28.5	30.2	27.4 ± 1.5
3	28.8	30.0	25.6	26.6	29.5	31.0	28.6 ± 0.8	29.3	33.1	23.9	23.7	29.8	31.0	28.5 ± 1.6
4	27.9	30.2	26.5	27.8	29.9	31.1	28.9 ± 0.7	29.3	33.8	24.3	24.0	30.2	31.1	28.8 ± 1.6
5	29.3	31.1	24.0	28.5	30.4	31.6	29.2 ± 1.1	28.7	33.8	24.4	24.7	30.8	31.4	29.0 ± 1.5
6	29.0	32.0	22.9	29.1	31.0	32.4	29.4 ± 1.4	28.5	33.8	25.2	25.2	31.6	32.4	29.5 ± 1.5
7	29.9	32.7	22.4	29.0	32.0	33.0	29.8 ± 1.6	29.8	34.7	26.2	25.4	32.2	33.3	30.3 ± 1.6
8	30.3	32.9	22.7	28.6	32.2	33.1	30.0 ± 1.6	30.2	35.1	26.4	25.2	33.1	33.3	30.6 ± 1.6
9	30.8	33.3	23.6	27.0	33.0	33.7	30.2 ± 1.7	30.0	35.8	26.9	25.7	34.0	33.6	31.0 ± 1.7
10	30.6	33.8	24.4	29.8	33.7	35.3	31.3 ± 1.6	30.5	36.7	28.2	27.3	35.4	35.6	32.3 ± 1.7
11	30.3	34.7	25.2	29.7	34.5	35.0	31.6 ± 1.6	30.5	37.0	29.1	27.2	35.8	35.4	32.5 ± 1.7
12	31.4	34.9	26.1	29.7	35.1	35.8	32.2 ± 1.6	31.6	37.9	29.8	27.9	36.5	36.3	33.3 ± 1.7
13	31.5	35.6	26.3	31.1	35.3	36.3	32.7 ± 1.6	32.0	37.6	29.5	28.8	36.8	36.1	33.5 ± 1.6
14	32.9	36.2	27.3	32.2	35.9	36.5	33.5 ± 1.4	32.5	38.1	30.4	28.1	37.5	35.9	33.8 ± 1.7
15	33.6	36.7	28.5	32.2	36.5	37.2	34.1 ± 1.4	33.6	38.9	29.5	29.4	38.3	36.6	34.4 ± 1.7
16	34.0	36.5	29.0	33.1	37.0	37.6	34.5 ± 1.3	33.8	39.1	30.2	30.3	39.2	36.1	34.8 ± 1.7
17	33.6	38.5	29.3	32.2	38.3	39.5	35.2 ± 1.7	34.0	40.6	29.3	29.0	41.6	39.9	35.7 ± 2.3
18	33.3	38.2	29.0	30.8	37.8	40.6	35.0 ± 1.9	34.1	40.8	30.2	27.2	40.5	39.7	35.4 ± 2.4
19	32.2	37.1	28.2	31.2	37.2	39.9	34.3 ± 1.8	34.4	39.5	29.5	25.9	39.2	38.1	34.4 ± 2.3
20	31.1	37.5	28.6	30.4	36.5	39.7	34.0 ± 1.8	33.3	38.9	30.4	24.0	38.0	37.6	33.7 ± 2.4

Appendix 1: Table 35

Experiment 2: Dry Matter Intake (g/day) of Fluke-infected and Control Sheep

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - -1	1062	1138	864	792	1070	1115	1007 ± 58	1078	1140	869	792	1107	1154	1023 ± 63
-1 - 0	1064	1153	956	822	1107	1151	1042 ± 53	1084	1154	971	826	1109	1157	1050 ± 53
0 - 1	941	1156	921	835	1121	1150	1021 ± 57	941	1156	923	835	1134	1144	1022 ± 57
1 - 2	1054	1151	928	882	1152	1157	1054 ± 50	1054	1151	928	882	1152	1157	1054 ± 50
2 - 3	1037	1149	943	829	1131	1157	1041 ± 54	1062	1157	952	829	1136	1157	1049 ± 54
3 - 4	933	1115	821	786	1111	1157	987 ± 66	935	1125	911	786	1121	1157	1006 ± 61
4 - 5	913	1146	688	899	1118	1157	987 ± 76	913	1153	891	899	1118	1157	1022 ± 54
5 - 6	887	1063	781	880	1113	1154	980 ± 61	887	1154	886	880	1116	1157	1013 ± 58
6 - 7	998	1144	691	855	1090	1156	989 ± 75	998	1157	678	855	1096	1157	990 ± 78
7 - 8	878	1131	616	544	1098	1119	898 ± 108	878	1131	616	544	1098	1120	989 ± 108
8 - 9	702	1131	719	673	1073	1123	904 ± 92	721	1131	719	673	1073	1123	907 ± 91
9 - 10	718	1131	770	815	1124	1131	948 ± 82	718	1131	770	815	1124	1131	948 ± 82
10 - 11	728	1122	777	869	1131	1131	960 ± 78	728	1131	777	869	1131	1131	961 ± 78
11 - 12	819	1131	865	876	1131	1131	992 ± 63	819	1131	865	876	1131	1131	992 ± 63
12 - 13	928	1131	811	1029	1131	1131	1027 ± 54	928	1131	811	1029	1131	1131	1027 ± 54
13 - 14	1031	1131	858	1020	1131	1131	1050 ± 44	1031	1131	858	1020	1131	1131	1050 ± 44
14 - 15	1038	1131	764	974	1131	1131	1028 ± 59	1028	1131	764	974	1131	1131	1028 ± 59
15 - 16	954	1131	802	1016	1131	1131	1028 ± 54	954	1131	802	1016	1131	1131	1028 ± 54
16 - 17	507	882	523	360	760	801	639 ± 83	507	1002	583	278	991	886	708 ± 121
17 - 18	660	903	537	476	781	813	695 ± 68	681	997	463	435	791	874	707 ± 92
18 - 19	618	930	561	484	722	771	681 ± 66	642	954	566	442	727	964	716 ± 86
19 - 20	637	952	622	450	716	858	706 ± 73	652	952	734	318	716	1036	735 ± 103

Appendix 1: Table 36

Experiment 2: Crude Protein Intakes (g/day) of Fluke-infected and Control Sheep

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - -1	141	151	115	105	142	148	134 ± 8	143	152	115	105	147	153	136 ± 8
-1 - 0	141	153	127	109	147	153	138 ± 7	144	153	129	110	147	154	140 ± 7
0 - 1	125	154	122	110	149	153	136 ± 8	125	154	123	110	151	152	136 ± 8
1 - 2	140	153	123	117	153	154	140 ± 7	140	153	123	117	153	154	140 ± 7
2 - 3	138	153	126	111	151	154	139 ± 7	141	154	127	111	152	154	140 ± 7
3 - 4	125	149	110	104	148	154	132 ± 9	125	150	122	104	149	154	134 ± 8
4 - 5	121	152	91	119	149	154	131 ± 10	121	153	118	119	149	154	136 ± 7
5 - 6	118	141	104	117	148	153	130 ± 8	118	153	118	117	148	154	135 ± 8
6 - 7	133	152	92	114	145	154	132 ± 10	133	154	90	114	146	154	132 ± 10
7 - 8	137	176	96	85	171	174	140 ± 17	137	176	96	85	171	175	140 ± 17
8 - 9	116	187	119	111	177	186	149 ± 15	119	187	119	111	177	186	150 ± 15
9 - 10	118	186	127	134	185	186	156 ± 13	118	186	127	134	185	186	156 ± 13
10 - 11	120	185	128	143	186	186	158 ± 13	120	186	128	143	186	186	158 ± 13
11 - 12	135	186	142	144	186	186	163 ± 10	135	186	142	144	186	186	163 ± 10
12 - 13	153	186	133	169	186	186	169 ± 9	153	186	133	169	186	186	169 ± 9
13 - 14	186	186	141	168	186	186	176 ± 8	186	186	141	168	186	186	176 ± 8
14 - 15	171	186	126	160	186	186	169 ± 10	171	186	126	160	186	186	169 ± 10
15 - 16	157	186	132	167	186	186	169 ± 9	157	186	132	167	186	186	169 ± 9
16 - 17	40	70	41	29	60	63	51 ± 7	40	79	46	22	78	70	56 ± 10
17 - 18	52	72	43	38	62	64	55 ± 5	54	79	37	34	63	69	56 ± 7
18 - 19	49	74	44	38	57	61	54 ± 5	51	76	45	35	58	76	57 ± 7
19 - 20	50	75	49	36	57	68	56 ± 6	52	75	58	25	57	82	58 ± 8

APPENDIX 2

Data relevant to Chapter 2:

The Relationship of Host Nutrition and Fascioliasis:

The aetiology of the anaemia which develops in sheep following infection with F. hepatica

Appendix 2. Table 1

Experiment 1: Blood Volumes 6 Weeks Before Infection with F. hepatica

Diet	Sheep No.	Plasma Volume ml	Plasma Volume ml/kg	Red Cell Volume ml	Red Cell Volume ml/kg	Blood Volume ml	Blood Volume ml/kg
Hay Only	Control						
	3	1313	37.8	647	18.6	1960	56.5
	6	1209	35.8	623	18.4	1832	54.2
	962	1192	34.8	614	17.9	1806	52.7
	Mean	1238	36.1	628	18.3	1866	54.5
	S.E.	38	0.9	10	0.2	48	1.1
	Infected						
	989	1123	34.2	605	18.4	1728	52.7
	981	1165	37.2	499	15.9	1664	53.2
	961	1229	34.3	691	19.3	1920	53.6
	Mean	1172	35.2	598	17.9	1771	53.2
	S.E.	31	1.0	56	1.0	77	0.3
	* 985	1126	35.0	555	17.2	1681	52.2
	* 992	1180	34.8	608	17.9	1788	52.7
Hay + Compound Diet	Control						
	7	1299	38.9	557	16.7	1856	55.6
	952	1345	38.9	633	18.3	1978	57.2
	964	1304	36.0	672	18.6	1976	54.6
	Mean	1316	37.9	621	17.9	1937	55.8
	S.E.	15	1.0	34	0.6	40	0.8
	Infected						
	5	1176	35.1	784	23.4	1960	58.5
	994	1217	37.0	534	16.2	1751	53.2
	965	1190	36.0	510	15.4	1700	51.4
	Mean	1194	36.0	609	18.3	1804	54.4
	S.E.	12	0.5	88	2.5	80	2.1
	* 954	1301	40.5	585	18.2	1886	58.8
	* 4	1223	36.1	718	21.2	1941	57.3

* Infected sheep without pair-fed control partners.

Appendix 2. Table 2

Experiment 1: Blood Volumes at the Time of Infection with F. hepatica

Diet	Sheep No.	Plasma Volume ml	Plasma Volume ml/kg	Red Cell Volume ml	Red Cell Volume ml/kg	Blood Volume ml	Blood Volume ml/kg
Hay Only	Control						
	3	1428	41.3	489	14.1	1917	55.4
	6	1260	41.3	490	16.1	1750	57.4
	962	1297	38.3	456	13.5	1753	51.7
	Mean	1328	40.3	478	14.6	1807	54.8
	S.E.	51	1.0	11	0.8	55	1.7
	Infected						
	989	1185	34.5	484	14.1	1669	48.7
	981	1215	40.9	449	15.1	1664	56.0
	961	1252	37.9	589	17.8	1841	55.8
	Mean	1217	37.8	507	15.7	1725	53.5
	S.E.	19	1.8	42	1.1	58	2.4
	* 985	1289	39.9	430	13.2	1719	52.9
	* 992	1319	37.4	513	14.5	1832	51.9
Hay + Compound Diet	Control						
	7	1301	34.3	558	14.7	1859	49.1
	952	1522	43.2	622	17.7	2144	60.9
	964	1504	41.5	542	15.0	2046	56.5
	Mean	1442	39.7	574	15.8	2016	55.5
	S.E.	71	2.7	24	1.0	84	3.4
	Infected						
	5	1338	35.8	615	16.4	1953	52.2
	994	1482	40.3	548	14.9	2030	55.2
	965	1489	41.6	523	14.6	2012	56.2
	Mean	1436	39.2	562	15.3	1998	54.5
	S.E.	49	1.8	27	0.6	23	1.2
	* 954	1365	39.8	558	16.3	1923	56.1
	* 4	1462	39.2	597	16.0	2059	55.2

* Infected sheep without pair-fed control partners.

Appendix 2. Table 3

Experiment 1: Blood Volumes 7 Weeks After Infection with F. hepatica

Diet	Sheep No.	Plasma Volume ml	Plasma Volume ml/kg	Red Cell Volume ml	Red Cell Volume ml/kg	Blood Volume ml	Blood Volume ml/kg
Hay Only	Control						
	3	1393	42.1	515	15.6	1908	57.6
	6	1310	44.1	369	12.4	1679	56.5
	962	1274	39.6	471	14.6	1745	54.2
	Mean	1326	41.9	452	14.2	1777	56.1
	S.E.	35	1.3	75	0.9	68	1.0
	Infected						
	989	1577	50.1	394	12.5	1971	62.6
	981	1462	51.1	366	12.8	1828	63.9
	961	1532	50.7	407	13.5	1939	64.2
	Mean	1524	50.6	389	12.9	1913	63.6
	S.E.	33	0.3	12	0.3	43	0.5
	* 985	1330	47.5	397	14.2	1727	61.7
	* 992	1489	42.8	510	14.7	1999	57.4
Hay + Compound Diet	Control						
	7	1354	38.5	553	15.7	1907	54.2
	952	1530	42.6	566	15.8	2096	58.4
	964	1479	42.3	604	17.3	2083	59.5
	Mean	1454	41.1	574	16.3	2029	57.4
	S.E.	52	1.3	15	0.5	61	1.6
	Infected						
	5	1486	42.2	578	16.4	2064	58.6
	994	1546	41.4	488	13.1	2034	54.5
	965	1489	42.9	496	14.3	1985	57.2
	Mean	1507	42.2	521	14.6	2028	56.8
	S.E.	20	0.4	29	1.0	23	1.2
	* 954	1442	45.9	431	13.7	1873	59.6
	* 4	1514	43.5	505	14.5	2019	58.0

* Infected sheep without pair-fed control partners.

Appendix 2. Table 4

Experiment 1: Blood Volumes 10 Weeks After Infection with F. hepatica

Diet	Sheep No.	Plasma Volume ml	Plasma Volume ml/kg	Red Cell Volume ml	Red Cell Volume ml/kg	Blood Volume ml	Blood Volume ml/kg
Hay Only	Control						
	3	1401	44.2	545	17.2	1946	61.4
	6	1284	42.2	352	11.6	1636	53.8
	962	1258	41.4	454	14.9	1712	56.3
	Mean	1314	42.6	450	14.6	1765	57.2
	S.E.	44	0.8	56	1.6	93	2.2
	Infected						
	989	1438	49.9	274	9.5	1712	59.4
	981	1590	57.4	349	12.6	1939	70.0
	961	1640	59.0	360	12.9	2000	71.9
	Mean	1556	55.4	328	11.7	1884	67.1
	S.E.	61	2.8	27	1.1	88	3.9
	* 985	1576	55.5	445.	15.7	2021	71.2
	* 992	1594	46.9	374	11.0	1968	57.9
Hay + Compound Diet	Control						
	7	1291	36.6	527	14.9	1818	51.5
	952	1473	42.1	545	15.6	2018	57.7
	964	1493	42.7	581	16.6	2074	59.3
	Mean	1419	40.5	551	15.7	1970	56.2
	S.E.	64	1.9	16	0.5	78	2.4
	Infected						
	5	1624	47.5	527	15.4	2151	62.9
	994	1773	49.7	389	10.9	2162	60.6
	965	1783	54.2	418	12.7	2201	66.9
	Mean	1727	50.5	445	13.0	2171	63.5
	S.E.	51	2.0	42	1.3	15	1.8
	* 954	1777	56.8	417	13.3	2194	70.1
	* 4	1770	51.2	456	13.2	2226	64.3

* Infected sheep without pair-fed control partners.

Appendix 2. Table 5

Experiment 1: Blood Volumes, 14 weeks after Infection with F. hepatica

Diet	Sheep No.	Plasma Volume ml	Plasma Volume ml/kg	Red Cell Volume ml	Red Cell Volume ml/kg	Blood Volume ml	Blood Volume ml/kg
Hay Only	Control						
	3	1297	46.3	492	17.6	1789	63.9
	6	1258	45.6	376	13.6	1634	59.2
	962	1324	45.7	418	16.0	1742	60.1
	Mean	1293	45.9	429	15.7	1722	61.1
	S.E.	19	0.2	34	1.2	46	1.4
	Infected						
	989	1398	51.8	138	5.1	1536	56.9
	981	1503	56.7	186	7.0	1689	63.7
	961	1662	61.1	271	10.0	1933	71.1
	Mean	1521	56.5	198	7.4	1719	63.9
	S.E.	77	2.7	39	1.4	116	4.1
	* 985	1405	57.1	174	7.1	1579	64.2
	* 992	1678	49.9	262	7.8	1940	57.7
Hay + Compound Diet	Control						
	7	1402	38.4	519	14.2	1921	52.6
	952	1508	42.6	530	15.0	2038	57.6
	964	1474	42.2	545	15.6	2019	57.9
	Mean	1461	41.1	531	14.9	1993	56.0
	S.E.	31	1.3	8	0.4	36	1.7
	Infected						
	5	1721	50.9	514	15.2	2235	66.1
	994	1915	52.2	351	9.6	2266	61.7
	965	1741	55.3	283	9.0	2024	64.3
	Mean	1792	52.8	383	11.3	2175	64.0
	S.E.	62	1.3	69	2.0	76	1.3
	* 954	1780	58.4	365	12.0	2145	70.3
	* 4	1873	51.7	384	10.6	2257	62.3

* Infected sheep without pair-fed control partners.

Appendix 2 Table 6

Experiment 1: Blood Volumes, 17 weeks after infection with F. hepatica

Diet	Sheep No.	Plasma Volume ml	Plasma Volume ml/kg	Red Cell Volume ml	Red Cell Volume ml/kg	Blood Volume ml	Blood Volume ml/kg
Hay + Compound Diet	Control						
	7	1384	38.3	486	13.5	1870	51.8
	952	1416	44.7	498	13.7	1914	60.4
	964	1474	41.3	518	14.5	1992	55.8
	Mean	1425	41.4	501	14.6	1925	56.0
	S.E.	26	1.8	9	0.6	36	2.5
	Infected						
	5	1608	48.1	353	10.6	1961	58.7
	994	1785	47.6	198	5.3	1983	52.9
	965	1755	56.1	274	8.8	2029	64.8
	Mean	1716	50.6	275	8.2	1991	58.8
	S.E.	55	2.8	45	1.6	20	3.4
	* 954	1716	55.4	303	9.8	2019	65.1
	* 4	1801	51.6	269	7.7	2070	59.3

* Infected sheep without pair-fed control partners.

Appendix 2 Table 7

Experiment 1: Blood Volumes, 20 weeks after infection with F. hepatica

Diet	Sheep No.	Plasma Volume		Red Cell Volume		Blood Volume	
		ml	ml/kg	ml	ml/kg	ml	ml/kg
Hay + Compound Diet	Control						
	7	1414	39.8	523	14.7	1937	54.6
	† 952	-	-	-	-	-	-
	964	1498	46.4	513	15.8	2011	61.9
	Mean	1456	43.1	518	15.3	1974	58.3
	S.E.	42	3.3	5	0.5	37	3.6
	Infected						
	5	1629	50.6	310	9.9	1939	61.9
	† 994	-	-	-	-	-	-
	965	1638	54.6	202	6.7	1840	60.7
	Mean	1634	52.6	256	8.3	1890	61.3
	S.E.	5	2.0	54	1.6	50	0.6
	* 954	1644	54.4	279	9.2	1923	63.4
	* 4	1834	57.3	204	6.4	2038	63.7

* Infected sheep without pair-fed partners.

† 994 died before the measurement and 952 killed at the same time.

Appendix 2. Table 8

Experiment 1: Faecal Blood Clearance (ml/day) in Control and Fluke-infected Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
Pre	4.9	3.7	7.6	5.4 ± 1.2	4.8	4.2	5.6	4.9 ± 0.4	5.1	8.8
0 - 2	2.3	2.5	5.8	3.5 ± 1.1	3.6	2.8	3.7	3.4 ± 0.3	3.6	5.2
2 - 4	2.4	2.1	3.0	2.5 ± 0.3	2.8	2.3	3.4	2.8 ± 0.3	2.3	3.1
4 - 7	2.7	1.8	2.3	2.3 ± 0.2	1.8	2.1	3.0	2.3 ± 0.4	1.8	2.5
7 - 8	32.1	12.2	17.8	20.7 ± 5.9	14.6	7.1	6.1	9.3 ± 2.7	5.1	19.3
8 - 9	12.8	5.7	4.4	7.6 ± 2.6	35.7	6.7	8.7	17.0 ± 9.4	2.9	25.7
9 - 10	5.0	2.8	2.6	3.5 ± 0.8	52.6	28.6	21.1	34.1 ± 9.5	4.6	52.2
10 - 11	2.6	1.7	1.4	1.9 ± 0.4	88.1	46.2	39.2	57.8 ±15.3	17.1	77.0
11 - 12	2.1	1.3	1.3	1.6 ± 0.3	129.7	81.5	66.8	92.7 ±19.0	38.1	107.2
12 - 13	2.1	1.3	1.2	1.5 ± 0.3	162.1	123.2	87.8	124.4 ±21.5	59.5	136.7
13 - 14	1.5	1.4	1.4	1.4 ± 0.0	212.5	172.8	112.5	165.9 ±29.1	98.2	167.7

* Infected sheep without pair-fed control partners.

Appendix 2. Table 9

Experiment 1: Faecal Blood Clearance (ml/day)
in Control and Fluke-infected Sheep Fed Hay plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
Pre	3.6	6.8	8.1	6.2 ± 1.3	6.1	7.8	5.7	6.5 ± 0.6	3.1	5.0
0 - 2	2.7	4.4	4.8	4.0 ± 0.6	4.2	4.5	2.8	3.8 ± 0.5	2.2	3.1
2 - 4	2.1	2.3	3.0	2.5 ± 0.3	3.0	2.2	2.4	2.5 ± 0.2	2.6	2.8
4 - 7	1.0	3.4	2.0	2.1 ± 0.7	2.2	1.2	2.1	1.8 ± 0.3	3.8	2.4
7 - 8	12.8	15.0	22.7	16.8 ± 3.0	8.9	19.2	8.7	12.3 ± 3.5	7.0	5.3
8 - 9	6.8	7.4	9.2	7.8 ± 0.7	5.5	34.9	10.0	16.8 ± 9.1	5.6	7.4
9 - 10	3.3	3.8	3.5	3.5 ± 0.1	5.2	73.5	19.0	32.6 ± 20.9	7.1	15.2
10 - 11	1.9	2.3	2.2	2.1 ± 0.1	13.4	102.9	17.8	44.7 ± 29.1	11.1	41.4
11- 12	1.5	1.9	1.7	1.7 ± 0.1	33.4	134.7	40.6	69.6 ± 32.6	21.3	75.5
12 - 13	1.5	2.4	1.7	1.9 ± 0.3	42.9	163.5	59.3	88.6 ± 37.8	35.2	103.5
13 - 14	1.9	1.4	1.4	1.6 ± 0.1	63.4	218.2	66.1	115.9 ± 51.2	48.8	127.6
14 - 15	9.4	14.8	12.1	12.1 ± 1.6	110.1	292.1	88.7	163.6 ± 64.5	84.9	198.7
15 - 16	4.2	5.5	5.9	5.2 ± 0.5	131.4	333.7	119.0	194.7 ± 69.6	119.7	239.2
16 - 17	3.2	3 .0	3.6	3.3 ± 0.2	147.2	294.4	153.5	198.4 ± 49.0	135.8	277.0
17 - 18	13.8	Dead	10.6	12.2 ± 1.6	195.3	Dead	159.0	177.2 ± 18.1	160.2	278.7
18 - 19	4.2		3.7	4.0 ± 0.2	253.1		261.3	257.2 ± 4.1	216.4	285.0
19 - 20	2.3		2.1	2.2 ± 0.1	256.1		303.9	280.0 ± 23.9	260.6	286.7

* Infected sheep without pair-fed control partners.

Appendix 2. Table 10

Experiment 1: Faecal Red Cell Clearance (ml/day) In Control and Fluke-infected Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
Pre	1.54	1.13	2.22	1.63 ± 0.30	1.33	1.26	1.54	1.38 ± 0.08	1.36	2.65
0 - 2	0.62	0.75	1.56	0.98 ± 0.28	1.00	0.73	0.99	0.91 ± 0.08	0.86	1.40
2 - 4	0.68	0.50	0.86	0.68 ± 0.10	0.74	0.53	0.78	0.68 ± 0.07	0.64	0.87
4 - 7	0.73	0.41	0.65	0.60 ± 0.09	0.44	0.46	0.69	0.53 ± 0.08	0.44	0.72
7 - 8	9.86	2.86	4.35	5.69 ± 2.13	2.92	1.42	1.28	1.87 ± 0.52	1.12	4.63
8 - 9	3.32	1.31	1.20	1.94 ± 0.69	6.43	1.27	1.65	3.12 ± 1.66	0.64	5.91
9 - 10	1.35	0.62	0.73	0.90 ± 0.23	8.94	4.35	3.80	5.70 ± 1.63	1.01	10.44
10 - 11	0.73	0.41	0.43	0.52 ± 0.10	15.96	7.48	5.98	9.81 ± 3.11	3.02	15.14
11 - 12	0.57	0.38	0.39	0.45 ± 0.05	20.82	13.03	10.13	14.66 ± 3.19	5.56	20.43
12 - 13	0.59	0.32	0.36	0.42 ± 0.08	22.74	17.91	12.76	17.80 ± 2.88	8.35	23.38
13 - 14	0.44	0.39	0.38	0.40 ± 0.00	23.43	20.76	15.80	20.00 ± 2.24	12.37	24.31

* Infected sheep without pair-fed control partners.

Appendix 2. Table 11

Experiment 1: Faecal Red Cell Clearance (ml/day) In Control and Fluke-infected Sheep Fed Hay plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
Pre	0.99	1.90	2.43	1.77 ± 0.42	2.07	2.11	1.45	1.88 ± 0.21	0.90	1.45
0 - 2	0.78	1.18	1.37	1.11 ± 0.17	1.30	1.12	0.76	1.06 ± 0.16	0.65	0.83
2 - 4	0.56	0.97	0.99	0.84 ± 0.14	0.96	0.55	0.81	0.77 ± 0.12	0.72	0.77
4 - 7	0.29	0.92	0.56	0.59 ± 0.18	0.62	0.28	0.80	0.57 ± 0.15	0.89	0.60
7 - 8	3.50	3.62	6.53	4.55 ± 0.99	2.23	4.13	1.98	2.78 ± 0.68	1.43	1.63
8 - 9	1.93	1.91	2.61	2.15 ± 0.23	1.44	7.26	2.12	3.61 ± 1.84	1.21	1.74
9 - 10	0.89	0.97	0.97	0.94 ± 0.00	1.30	13.61	3.76	6.22 ± 3.76	1.39	3.32
10 - 11	0.52	0.63	0.71	0.62 ± 0.04	2.92	18.06	3.23	8.07 ± 5.00	2.18	8.13
11 - 12	0.41	0.50	0.58	0.50 ± 0.04	6.98	22.87	6.12	11.99 ± 5.45	3.92	14.36
12 - 13	0.38	0.58	0.56	0.51 ± 0.05	9.39	26.74	8.64	14.92 ± 5.91	6.56	17.62
13 - 14	0.50	0.44	0.42	0.45 ± 0.00	13.94	34.89	9.28	19.37 ± 7.88	8.34	21.70
14 - 15	2.53	3.99	4.72	3.75 ± 0.64	24.23	40.23	13.38	25.95 ± 7.80	13.08	32.53
15 - 16	1.12	1.43	2.27	1.61 ± 0.34	25.00	45.38	17.12	29.17 ± 8.42	19.00	34.18
16 - 17	0.86	0.81	1.74	1.14 ± 0.30	27.23	38.62	21.18	29.01 ± 5.11	21.33	36.93
17 - 18	3.57	Dead	2.65	3.11 ± 0.46	32.20	Dead	19.93	26.07 ± 6.13	24.83	33.44
18 - 19	1.13		0.96	1.05 ± 0.08	40.50		30.10	35.30 ± 5.20	32.46	31.35
19 - 20	0.60		0.55	0.58 ± 0.00	39.71		31.92	35.82 ± 3.89	36.48	30.10

* Infected sheep without pair-fed control partners.

Appendix 2. Table 12

Experiment 1: Ferrokinetic Indices Before Infection with F. hepatica

Diet	Sheep No.	Serum Iron µg%	^{59}Fe Half-Life mins	Plasma Iron Turnover Rate		
				mg/day	mg/day/kg	mg/day/100ml blood
Hay Only	Control					
	3	156	153	14.53	0.420	0.758
	6	138	143	12.13	0.398	0.693
	962	142	188	9.78	0.288	0.558
	Mean	145	161	12.15	0.369	0.670
	S.E.	5	14	1.37	0.041	0.059
	Infected					
	989	140	184	9.00	0.262	0.539
	981	133	129	12.50	0.421	0.751
	961	169	145	10.11	0.306	0.549
	Mean	147	153	10.54	0.330	0.613
	S.E.	11	16	1.03	0.047	0.069
	* 985	154	202	9.81	0.302	0.571
	* 992	146	174	11.04	0.313	0.603
Hay + Compound Diet	Control					
	7	124	152	10.59	0.279	0.570
	952	140	138	15.41	0.438	0.719
	964	140	185	11.36	0.314	0.555
	Mean	135	158	12.45	0.344	0.615
	S.E.	5	14	1.49	0.048	0.052
	Infected					
	5	183	202	12.10	0.323	0.620
	994	130	179	10.74	0.292	0.529
	965	154	156	14.67	0.410	0.729
	Mean	156	179	12.50	0.342	0.626
	S.E.	15	13	1.15	0.035	0.058
	* 954	143	150	12.99	0.379	0.676
	* 4	160	212	11.01	0.295	0.535

* Infected sheep without pair-fed control partners

Appendix 2. Table 13

Experiment 1: Ferrokinetic Indices 7 Weeks After Infection with F. hepatica

Diet	Sheep No.	Serum Iron µg%	^{59}Fe Half-Life mins	Plasma Iron Turnover Rate		
				mg/day	mg/day/kg	mg/day/100ml blood
Hay Only	Control					
	3	153	206	10.32	0.312	0.541
	6	124	156	10.39	0.350	0.618
	962	131	165	10.09	0.314	0.578
	Mean	136	176	10.27	0.325	0.579
	S.E.	9	15	0.09	0.012	0.022
	Infected					
	989	145	68	33.56	1.065	1.702
	981	128	85	21.97	0.768	1.202
	961	124	102	18.59	0.615	0.958
	Mean	132	85	24.71	0.816	1.287
	S.E.	6	10	4.53	0.132	0.219
	* 985	134	112	15.88	0.567	0.920
	* 992	134	77	25.86	0.743	1.294
Hay + Compound Diet	Control					
	7	141	173	11.01	0.313	0.577
	952	131	182	10.99	0.306	0.524
	964	149	180	12.22	0.349	0.587
	Mean	140	178	11.41	0.323	0.563
	S.E.	5	3	0.41	0.013	0.020
	Infected					
	5	165	162	15.10	0.429	0.732
	994	136	96	21.86	0.586	1.074
	965	137	147	13.85	0.399	0.698
	Mean	146	135	16.94	0.471	0.835
	S.E.	10	20	2.49	0.058	0.120
	* 954	137	159	12.40	0.395	0.662
	* 4	122	142	12.98	0.373	0.643

* Infected sheep without pair-fed control partners.

Appendix 2. Table 14

Experiment 1: Ferrokinetic Indices 14 Weeks after Infection with F. hepatica

Diet	Sheep No.	Serum Iron µg%	^{59}Fe Half-life mins	Plasma Iron Turnover Rate		
				mg/day	mg/day/kg	mg/day/100ml blood
Hay Only	Control					
	3	174	308	7.31	0.261	0.409
	6	157	240	8.21	0.298	0.503
	962	147	180	10.79	0.372	0.619
	Mean	159	243	8.77	0.310	0.510
	S.E.	8	37	1.04	0.033	0.061
	Infected					
	989	27	23	16.38	0.607	1.066
	981	38	28	20.36	0.768	1.205
	961	65	39	27.64	1.016	1.430
	Mean	43	30	21.46	0.797	1.234
	S.E.	11	5	3.30	0.119	0.106
	* 985	85	68	17.53	0.712	1.110
	* 992	69	30	38.51	1.146	1.985
Hay + Compound Diet	Control					
	7	179	196	12.78	0.350	0.665
	952	128	189	10.19	0.288	0.500
	964	146	208	10.32	0.296	0.511
	Mean	151	198	11.10	0.311	0.559
	S.E.	15	6	0.84	0.019	0.053
	Infected					
	5	169	92	31.55	0.933	1.412
	994	174	48	69.27	1.888	3.057
	965	181	89	35.33	1.122	1.745
	Mean	175	76	45.38	1.320	2.071
	S.E.	3	14	11.99	0.292	0.502
	* 954	130	75	30.79	1.010	1.436
	* 4	176	90	36.55	1.010	1.620

* Infected sheep without pair-fed control partners.

Appendix 2. Table 15

Experiment 1: Ferrokinetic Indices 17 weeks after Infection with F. hepatica

Diet	Sheep No.	Serum Iron µg%	^{59}Fe Half-life mins	Plasma Iron Turnover Rate		
				mg/day	mg/day/kg	mg/day/100ml blood
Hay + Compound Diet	Control					
	7	181	219	11.41	0.316	0.610
	952	144	274	7.43	0.234	0.388
	964	176	165	15.69	0.440	0.788
	Mean	167	219	11.51	0.330	0.595
	S.E.	12	31	2.38	0.060	0.116
	Infected					
	5	102	36	45.47	1.361	2.319
	994	47	17	49.25	1.313	2.485
	965	136	62	38.42	1.228	1.893
	Mean	95	38	44.38	1.301	2.232
	S.E.	26	13	3.17	0.039	0.176
	* 954	116	45	44.14	1.424	2.187
	* 4	185	36	92.36	2.646	4.461

* Infected sheep without pair-fed control partners.

Appendix 2. Table 16

Experiment 1: Ferrokinetic Indices 20 Weeks after Infection with F. hepatica

Diet	Sheep No.	Serum Iron µg%	^{59}Fe Half-Life mins	Plasma Iron Turnover Rate		
				mg/day	mg/day/kg	mg/day/100ml blood
Hay + Compound Diet	Control					
	7	138	222	8.77	0.247	0.453
	† 952	-	-	-	-	-
	964	164	159	15.42	0.474	0.767
	Mean	151	191	12.10	0.361	0.610
	S.E.	13	32	3.33	0.114	0.157
	Infected					
	5	67	38	28.66	0.916	1.478
	† 994	-	-	-	-	-
	965	136	57	39.00	1.287	2.119
	Mean	102	48	33.83	1.102	1.799
	S.E.	35	10	5.17	0.186	0.321
	* 954	123	39	51.74	1.713	2.690
	* 4	129	49	48.18	1.506	2.364

* Infected sheep without pair-fed control partners

† 994 died before the measurement and 952 was killed at the same time.

Appendix 2. Table 17

Experiment 1: Serum Iron Concentration (µg/100ml) in Control and Fluke-infected Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
0	156	138	142	145 ± 5	140	133	169	147 ± 11	154	146
1	134	160	146	147 ± 8	136	146	146	143 ± 3	126	128
2	150	150	133	144 ± 6	142	135	158	145 ± 7	139	100
3	149	166	168	161 ± 6	138	121	149	136 ± 8	130	112
4	171	151	162	161 ± 6	131	131	178	147 ± 16	131	140
5	167	161	179	169 ± 5	145	130	149	141 ± 6	140	134
6	153	149	154	152 ± 2	134	125	135	131 ± 3	123	124
7	153	124	131	136 ± 9	145	128	124	132 ± 6	134	134
8	147	133	151	144 ± 5	100	100	90	97 ± 5	94	118
9	158	138	138	145 ± 6	90	102	96	96 ± 3	84	102
10	146	140	157	148 ± 5	105	70	60	78 ± 14	87	132
11	131	122	143	132 ± 6	92	84	98	91 ± 4	102	125
12	174	140	147	154 ± 10	70	64	92	75 ± 9	85	103
13	153	144	133	143 ± 6	44	67	63	58 ± 7	93	95
14	174	157	147	159 ± 8	36	51	70	52 ± 10	102	43
15	178	168	138	161 ± 12	23	25	60	36 ± 12	85	51

* Infected sheep without pair-fed control partners.

Appendix 2. Table 18

Experiment 1: Serum Iron Concentration (µg/100ml) in Control and Fluke-infected Sheep Fed Hay plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
0	124	140	140	135 ± 5	183	130	154	156 ± 15	143	160
1	154	128	122	135 ± 10	169	112	136	139 ± 17	128	121
2	156	145	137	146 ± 6	201	122	139	154 ± 24	137	137
3	151	138	130	140 ± 6	166	121	130	139 ± 14	161	170
4	168	128	151	149 ± 12	205	133	147	162 ± 22	168	184
5	138	149	149	145 ± 4	206	130	151	162 ± 23	138	151
6	132	143	155	143 ± 7	179	142	136	152 ± 13	149	136
7	141	131	149	140 ± 5	165	136	137	146 ± 10	137	122
8	147	116	159	141 ± 13	155	117	116	129 ± 13	118	100
9	158	114	140	137 ± 13	140	106	121	122 ± 10	117	105
10	149	107	149	135 ± 14	126	113	108	116 ± 5	102	91
11	146	118	131	132 ± 8	149	112	86	116 ± 18	71	92
12	160	119	140	140 ± 12	126	191	89	135 ± 30	85	137
13	155	133	147	145 ± 6	172	179	126	159 ± 17	79	151
14	179	128	146	151 ± 15	169	206	181	185 ± 11	130	209
15	183	128	170	160 ± 17	165	174	196	178 ± 9	125	167
16	176	146	137	153 ± 12	169	183	159	170 ± 7	157	185
17	198	161	186	182 ± 11	102	61	136	100 ± 22	143	214
18	158	Dead	180	169 ± 11	94	Dead	178	136 ± 42	123	200
19	154		134	144 ± 10	56		168	112 ± 56	104	160
20	170		164	167 ± 3	67		136	102 ± 35	123	129

* Infected sheep without pair-fed control partners.

Appendix 2. Table 19

Experiment 1: Faecal Losses of Haemoglobin Iron (mg/day) in Control and Fluke-infected Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
Pre	1.47	1.18	2.01	1.55 ± 0.24	1.47	1.12	1.70	1.43 ± 0.17	1.14	2.62
0 - 2	0.68	0.79	1.65	1.04 ± 0.31	1.05	0.78	1.12	0.98 ± 0.10	0.73	1.56
2 - 4	0.71	0.57	0.93	0.74 ± 0.10	0.80	0.61	0.85	0.75 ± 0.07	0.62	0.91
4 - 7	0.82	0.48	0.70	0.67 ± 0.10	0.43	0.48	0.69	0.53 ± 0.08	0.41	0.74
7 - 8	9.76	3.16	5.47	6.13 ± 1.93	3.07	1.52	1.39	1.99 ± 0.54	1.01	5.03
8 - 9	4.01	1.46	1.35	2.27 ± 0.87	6.68	1.41	1.89	3.33 ± 1.68	0.61	6.05
9 - 10	1.57	0.70	0.77	1.01 ± 0.28	9.14	3.54	4.09	5.59 ± 1.78	0.93	11.16
10 - 11	0.79	0.43	0.40	0.54 ± 0.13	15.60	7.33	6.81	9.91 ± 2.85	2.97	15.69
11 - 12	0.63	0.33	0.39	0.45 ± 0.09	22.53	12.52	11.16	15.40 ± 3.58	5.98	21.84
12 - 13	0.64	0.34	0.35	0.44 ± 0.10	20.57	17.69	11.53	16.60 ± 2.67	13.69	23.97
13 - 14	0.44	0.36	0.39	0.40 ± 0.02	17.74	17.60	14.27	16.54 ± 1.13	11.15	22.68

* Infected sheep without pair-fed control partners.

Appendix 2. Table 20

Experiment 1: Faecal Losses of Haemoglobin Iron (mg/day) in Control and Fluke-infected Sheep Fed Hay plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
Pre	1.07	2.08	2.08	1.74 ± 0.34	1.89	2.10	1.58	1.86 ± 0.15	0.97	1.58
0 - 2	0.84	1.36	1.44	1.21 ± 0.19	1.37	1.23	0.80	1.13 ± 0.17	0.70	0.92
2 - 4	0.64	0.68	0.98	0.77 ± 0.11	1.01	0.60	0.73	0.78 ± 0.12	0.80	0.81
4 - 7	0.32	1.07	0.65	0.68 ± 0.22	0.70	0.34	0.62	0.55 ± 0.11	1.00	0.67
7 - 8	4.00	4.43	7.14	5.19 ± 0.98	2.71	4.96	2.31	3.33 ± 0.82	1.76	1.41
8 - 9	2.18	2.21	2.88	2.42 ± 0.23	1.63	8.19	1.05	3.62 ± 2.29	1.41	1.94
9 - 10	1.08	1.14	1.11	1.11 ± 0.02	1.41	13.49	4.33	6.41 ± 3.64	1.59	3.62
10 - 11	0.58	0.68	0.69	0.65 ± 0.04	3.32	18.89	3.55	8.59 ± 5.15	2.23	8.33
11 - 12	0.44	0.55	0.53	0.51 ± 0.03	8.18	24.73	6.76	13.22 ± 5.77	4.27	14.12
12 - 13	0.46	0.76	0.54	0.59 ± 0.09	10.65	28.41	9.23	16.10 ± 6.17	6.80	19.35
13 - 14	0.59	0.42	0.42	0.48 ± 0.06	15.52	35.58	10.32	20.47 ± 7.70	8.83	20.82
14 - 15	2.75	4.28	3.66	3.56 ± 0.44	23.94	41.70	14.22	26.62 ± 8.05	14.16	33.83
15 - 16	1.23	1.68	1.60	1.50 ± 0.14	25.39	43.15	18.21	28.92 ± 7.41	19.99	37.38
16 - 17	0.98	0.91	0.95	0.95 ± 0.02	26.49	31.00	19.45	25.65 ± 3.36	22.20	39.46
17 - 18	4.03	Dead	2.79	3.41 ± 0.62	33.48	Dead	17.08	25.28 ± 8.20	21.71	34.15
18 - 19	1.26		0.99	1.13 ± 0.14	43.37		29.20	36.29 ± 7.09	25.70	31.35
19 - 20	0.67		0.60	0.64 ± 0.04	42.08		33.08	37.58 ± 4.50	31.24	26.32

* Infected sheep without pair-fed control partners.

Appendix 2: Table 21

Experiment 1: Dietary Iron intake (mg/day) of Fluke-infected and Control Sheep fed Hay Only

Days After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
0 - 6	276	238	253	256 ± 11	248	211	264	241 ± 16	278	329
6 - 11	225	192	233	217 ± 13	250	206	232	229 ± 13	236	274
11 - 16	364	316	332	337 ± 14	376	322	348	349 ± 16	392	446
16 - 22	402	270	297	323 ± 40	415	333	335	361 ± 27	306	450
22 - 28	127	107	122	119 ± 6	119	104	124	116 ± 6	115	187
28 - 33	113	135	154	134 ± 12	109	138	158	135 ± 14	119	181
33 - 38	222	187	208	206 ± 10	216	230	207	218 ± 7	195	264
38 - 43	263	261	266	263 ± 1	265	263	272	267 ± 3	261	346
43 - 48	274	247	257	259 ± 8	277	250	260	262 ± 8	246	324
48 - 55	225	218	241	228 ± 7	218	217	222	219 ± 2	231	282
55 - 60	297	272	248	272 ± 14	299	397	240	312 ± 46	254	400
60 - 65	312	433	326	357 ± 38	326	437	331	365 ± 36	385	512
65 - 69	354	364	321	346 ± 13	352	348	333	344 ± 6	353	446
69 - 75	347	333	340	340 ± 4	344	332	355	344 ± 7	381	461
75 - 81	216	196	221	221 ± 8	211	197	205	204 ± 4	265	296
81 - 86	218	208	232	219 ± 7	216	203	227	216 ± 7	245	304
86 - 91	208	186	225	206 ± 11	204	180	221	202 ± 12	230	289
91 - 96	168	171	229	189 ± 20	165	169	236	190 ± 23	232	217
96 - 103	100	155	181	145 ± 24	95	153	188	145 ± 27	192	267
103 - 106		73	128	101 ± 28		60	132	96 ± 36		154

* Infected sheep without pair-fed control partners.

Appendix 2: Table 22

Experiment 1: Dietary Iron Intake (mg/day) of Fluke-infected and Control Sheep Fed Hay and Compound Diet

Days After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
0 - 6	389	368	361	373 ± 8	369	351	356	359 ± 5	365	357
6 - 11	359	368	322	350 ± 14	363	352	330	348 ± 10	314	311
11 - 16	425	487	423	445 ± 21	419	486	440	448 ± 20	376	372
16 - 22	426	441	439	435 ± 5	425	448	431	435 ± 7	371	383
22 - 28	254	281	267	267 ± 8	249	282	269	267 ± 10	231	230
28 - 33	260	312	299	290 ± 16	268	310	293	290 ± 12	259	244
33 - 38	319	362	324	335 ± 14	325	366	328	340 ± 13	315	289
38 - 43	357	449	343	383 ± 33	364	449	342	385 ± 33	362	362
43 - 48	353	424	337	371 ± 27	356	419	342	372 ± 24	330	338
48 - 55	323	354	291	323 ± 18	314	347	289	317 ± 17	305	338
55 - 60	370	424	348	381 ± 23	362	433	363	386 ± 24	356	356
60 - 65	404	466	401	424 ± 21	400	471	395	422 ± 25	380	455
65 - 69	370	455	368	398 ± 29	364	471	367	401 ± 35	370	396
69 - 75	349	449	304	367 ± 43	344	427	300	357 ± 37	374	334
75 - 81	269	371	258	299 ± 36	238	352	248	279 ± 36	254	298
81 - 86	323	363	263	316 ± 29	320	363	256	313 ± 31	252	379
86 - 91	348	354	257	320 ± 31	337	356	260	318 ± 29	338	374
91 - 96	339	333	265	312 ± 24	356	339	276	324 ± 24	325	355
96 - 103	336	326	271	311 ± 20	339	323	270	311 ± 21	298	340
103 - 110	263	247	239	250 ± 7	258	230	245	244 ± 8	249	252
110 - 115	280	204	257	247 ± 23	275	202	256	244 ± 22	266	254
115 - 122	262	73	234	190 ± 59	257	69	228	185 ± 58	259	245
122 - 127	269	Dead	220	245 ± 25	262	Dead	220	241 ± 21	254	229
127 - 132	258		200	229 ± 29	250		200	225 ± 25	238	207
132 - 137	254		167	211 ± 44	251		157	204 ± 47	249	162

* Infected sheep without pair-fed control partners.

Appendix 2. Table 23

Experiment 2: Blood Volumes 2 Weeks Before Infection with F. hepatica

Sheep No.	Plasma Volume		Red Cell Volume		Blood Volume	
	ml	ml/kg	ml	ml/kg	ml	ml/kg
Control						
70	1100	43.7	449	17.8	1549	61.5
51	1111	41.9	523	19.7	1634	61.7
80	996	44.5	437	19.5	1433	64.0
98	973	41.2	458	19.4	1431	60.6
83	1152	44.8	567	22.1	171 9	66.9
97	1288	46.5	649	23.4	1937	69.9
Mean	1103	43.8	514	20.3	1617	64.1
S.E.	47	0.8	34	0.8	79	1.5
Infected						
78	1135	42.8	585	22.1	1720	64.9
77	1217	42.6	534	18.7	1751	61.2
60	997	47.3	417	19.8	1414	67.0
68	1018	46.1	513	23.2	1531	69.3
54	1052	41.1	462	18.0	1514	59.1
61	1192	43.1	537	19.4	1732	62.5
Mean	1102	43.8	508	20.2	1610	64.0
S.E.	38	1.0	24	0.8	58	1.5

Appendix 2. Table 24

Experiment 2: Blood Volumes 4 weeks after Infection with F. hepatica

Sheep No.	Plasma Volume		Red Cell Volume		Blood Volume	
	ml	ml/kg	ml	ml/kg	ml	ml/kg
Control						
70	1353	48.5	553	19.8	1906	68.3
51	1342	44.4	548	18.1	1890	62.6
80	1120	43.2	515	19.4	1635	61.7
98	1302	46.8	532	19.1	1834	66.0
83	1294	43.3	623	20.8	1917	64.1
97	1391	44.7	596	19.2	1987	63.9
Mean	1300	45.2	561	19.4	1862	64.4
S.E.	39	0.9	17	0.4	50	1.0
Infected						
78	1276	43.5	560	19.1	1836	62.7
77	1463	43.3	598	17.7	2061	61.0
60	963	39.6	453	18.6	1416	58.3
68	1040	43.3	512	21.3	1552	64.7
54	1477	48.9	664	22.0	2141	70.9
61	1485	47.7	667	21.4	2152	69.2
Mean	1284	44.4	576	20.0	1860	64.5
S.E.	95	1.4	35	0.7	129	2.0

Appendix 2. Table 25

Experiment 2: Blood Volumes 8 Weeks After Infection with F. hepatica

Sheep No.	Plasma Volume		Red Cell Volume		Blood Volume	
	ml	ml/kg	ml	ml/kg	ml	ml/kg
Control						
70	1429	47.2	515	17.0	1944	64.2
51	1538	46.7	569	17.3	2107	64.0
80	1087	47.9	423	18.6	1510	66.5
98	1310	45.8	561	19.6	1871	65.4
83	1453	45.1	653	20.3	2106	65.4
97	1514	45.7	618	18.7	2132	64.4
Mean	1389	46.4	557	18.6	1945	65.0
S.E.	69	0.4	33	0.5	97	0.4
Infected						
78	1383	45.8	512	17.0	1894	62.7
77	1738	49.5	579	16.5	2317	66.0
60	1230	46.6	443	16.8	1673	63.4
68	1213	48.1	362	14.4	1575	62.5
54	1642	49.6	577	17.4	2219	67.0
61	1605	48.2	624	18.7	2229	66.9
Mean	1469	48.0	516	16.8	1985	64.8
S.E.	91	0.6	40	0.6	129	0.9

Appendix 2. Table 26

Experiment 2: Blood Volumes 12 Weeks After Infection with F. hepatica

Sheep No.	Plasma Volume		Red Cell Volume		Blood Volume	
	ml	ml/kg	ml	ml/kg	ml	ml/kg
Control						
70	1387	44.2	513	16.3	1900	60.5
51	1596	45.7	575	16.5	2171	62.2
80	1162	44.5	475	18.2	1637	62.7
98	1358	45.7	568	19.1	1926	64.8
83	1560	44.4	637	18.1	2197	62.6
97	1532	42.8	626	17.5	2158	60.3
Mean	1433	44.6	566	17.6	1998	62.2
S.E.	67	0.4	26	0.4	89	0.7
Infected						
78	1488	47.1	420	13.3	1908	60.4
77	1754	46.3	554	14.6	2308	60.9
60	1358	45.6	429	14.4	1787	60.0
68	1396	50.0	371	13.3	1767	63.3
54	1778	48.7	561	15.4	2339	64.1
61	1609	44.3	522	14.4	2131	58.7
Mean	1564	47.0	476	14.2	2040	61.2
S.E.	73	0.8	33	0.3	104	0.8

Appendix 2. Table 27

Experiment 2: Blood Volumes 16 Weeks After Infection with F. hepatica

Sheep No.	Plasma Volume		Red Cell Volume		Blood Volume	
	ml	ml/kg	ml	ml/kg	ml	ml/kg
Control						
70	1411	41.5	535	15.7	1946	57.2
51	1460	40.0	554	15.2	2014	55.2
80	1174	40.5	434	15.0	1608	55.4
98	1514	45.7	649	19.6	2163	65.3
83	1479	40.0	696	18.8	2175	58.8
97	1535	40.8	658	17.5	2193	58.3
Mean	1429	41.4	588	17.0	2017	58.4
S.E.	54	0.9	40	0.8	91	1.5
Infected						
78	1465	43.3	488	14.4	1953	57.8
77	1818	46.5	513	13.1	2330	59.6
60	1341	44.4	356	11.8	1697	56.2
68	1350	44.6	359	11.8	1709	56.4
54	1792	45.7	520	13.3	2312	59.0
61	1689	46.8	476	13.2	2165	60.0
Mean	1576	45.2	452	12.9	2028	58.2
S.E.	89	0.6	31	0.4	117	0.7

Appendix 2. Table 28

Experiment 2: Blood Volumes 20 Weeks After Infection with F. hepatica

Sheep No.	Plasma Volume		Red Cell Volume		Blood Volume	
	ml	ml/kg	ml	ml/kg	ml	ml/kg
Control						
70	1360	43.7	555	17.8	1915	61.6
51	1420	37.9	594	15.8	2014	53.7
80	1151	40.2	437	15.3	1588	55.5
98	1228	40.4	633	20.8	1861	61.2
83	1314	36.0	590	16.2	1904	52.2
97	1355	34.1	678	17.1	1993	50.2
Mean	1305	38.7	581	17.2	1879	55.7
S.E.	40	1.4	34	0.8	63	1.9
Infected						
78	1345	40.4	368	11.1	1713	51.4
77	1647	42.3	386	9.9	2033	52.3
60	1345	44.2	256	8.4	1601	52.7
68	1355	56.5	202	8.4	1557	64.9
54	1672	44.0	405	10.7	2077	54.7
61	1629	43.3	382	10.2	2011	53.5
Mean	1499	45.1	333	9.8	1832	54.9
S.E.	68	2.3	34	0.5	96	2.0

Appendix 2: Table 29

Experiment 2: Faecal Blood Clearance (ml/day) in Control and Fluke-infected Sheep

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - 2	5.8	7.1	4.2	4.6	8.2	6.2	6.0 ± 0.6	4.4	6.0	7.3	6.4	8.2	5.7	6.3 ± 0.5
4 - 5	3.7	6.2	N.D.	4.5	7.3	4.2	5.2 ± 0.7	3.2	5.4	5.4*	6.6	5.7	6.2	5.4 ± 0.6
5 -6	2.7	5.4	N.D.	3.3	6.7	5.6	4.7 ± 0.8	2.5	6.2	5.1*	5.0	7.5	4.1	5.1 ± 0.9
6 - 7	3.2	4.4	N.D.	3.1	6.3	5.0	4.4 ± 0.5	2.3	8.5	8.2*	5.1	15.0	10.3	8.2 ± 1.8
7 - 8	3.1	3.6	N.D.	2.8	6.4	4.7	4.1 ± 0.5	8.1	29.9	19.7*	22.4	30.8	7.1	19.7 ± 4.2
8 - 9	2.8	2.9	3.7	3.3	5.8	4.6	3.9 ± 0.5	8.8	56.1	54.4	40.8	57.7	19.7	39.6 ± 8.5
9 - 10	2.5	3.1	2.7	4.0	6.4	5.1	4.0 ± 0.6	5.7	59.5	55.7	54.8	65.6	72.4	52.3 ± 9.7
10 - 11	2.9	2.5	3.4	2.7	6.2	5.7	3.9 ± 0.7	11.0	57.2	45.8	97.7	113.9	71.1	66.1 ±15.1
11 - 12	2.4	2.6	4.2	3.0	5.8	5.4	3.9 ± 0.6	31.9	53.6	75.0	69.4	100.1	79.2	68.2 ± 9.5
12 - 13	3.1	2.4	3.7	3.0	6.2	4.3	3.8 ± 0.6	38.7	98.4	78.2	88.9	127.6	64.2	82.7 ±12.4
13 - 14	3.3	3.2	3.2	4.5	5.8	5.0	4.2 ± 0.5	53.8	104.2	78.8	125.5	174.9	83.9	103.5 ±17.4
14 - 15	2.7	2.8	3.3	4.6	5.4	5.5	4.1 ± 0.5	53.3	105.9	65.1	162.4	192.3	82.8	110.3 ±22.7
15 - 16	2.9	2.5	3.0	3.9	6.2	5.1	3.9 ± 0.6	62.9	73.2	41.4	169.5	195.1	74.3	102.7 ±25.8
16 - 17	0.9	1.7	2.6	2.4	4.1	4.4	2.7 ± 0.6	72.9	63.3	60.1	174.8	135.7	59.0	94.3 ±20.0
17 - 18	1.1	1.2	2.7	5.5	7.3	4.9	3.8 ± 1.0	59.8	45.8	147.4	167.2	134.6	82.8	106.3 ±20.5
18 - 19	1.5	2.1	3.5	3.3	2.5	5.5	3.1 ± 0.6	49.6	52.3	224.9	161.2	170.4	87.1	124.3 ±29.3
19 - 20	1.7	2.0	1.9	0.9	4.4	5.7	2.8 ± 0.8	46.3	56.0	132.1	245.6	183.4	98.8	127.0 ±31.4

N.D. not determined
* not included in statistical analysis

Appendix 2: Table 30

Experiment 2: Faecal Red Cell Clearance (ml/day) in Control and Fluke-infected Sheep

Weeks After Infection	Controls							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - 2	1.8	2.2	1.4	1.5	2.8	2.0	2.0 ± 0.2	1.5	1.8	2.4	2.2	2.6	1.8	2.1 ± 0.2
4 - 5	1.1	1.8	N.D.	1.3	2.4	1.3	1.6 ± 0.2	1.0	1.6	1.6*	2.2	1.8	1.9	1.7 ± 0.2
5 - 6	0.8	1.6	N.D.	1.0	2.0	1.7	1.4 ± 0.2	0.8	1.7	1.6*	1.5	2.3	1.2	1.5 ± 0.3
6 - 7	1.0	1.4	N.D.	1.0	2.0	1.6	1.4 ± 0.2	0.7	2.4	2.4*	1.5	4.4	2.9	2.4 ± 0.5
7 - 8	0.9	1.0	N.D.	0.9	2.0	1.5	1.3 ± 0.2	2.1	7.9	5.3*	5.9	8.9	1.9	5.3 ± 1.2
8 - 9	0.7	0.8	1.0	1.0	1.8	1.3	1.1 ± 0.2	2.4	14.0	14.5	9.4	15.0	5.5	10.1 ± 2.2
9 - 10	0.7	0.9	0.8	1.3	1.9	1.5	1.2 ± 0.2	1.4	14.0	13.4	10.4	16.4	18.1	12.3 ± 2.4
10 - 11	0.9	0.8	1.0	0.8	1.8	1.7	1.2 ± 0.2	2.8	12.6	11.0	21.0	29.6	17.8	15.8 ± 3.8
11 - 12	0.7	0.8	1.2	0.9	1.7	1.7	1.2 ± 0.2	7.5	12.9	18.0	15.3	24.0	18.6	16.1 ± 2.3
12 - 13	0.8	0.6	1.1	0.9	1.8	1.3	1.1 ± 0.2	8.5	23.6	18.8	18.7	30.6	15.7	19.3 ± 3.0
13 - 14	1.0	0.9	0.9	1.3	1.6	1.4	1.2 ± 0.1	11.8	22.9	17.3	25.7	42.0	19.7	23.2 ± 4.2
14 - 15	0.8	0.8	1.0	1.4	1.6	1.8	1.2 ± 0.2	12.5	27.0	15.0	35.7	47.1	20.7	26.3 ± 5.4
15 - 16	0.9	0.7	0.8	1.2	1.9	1.5	1.2 ± 0.2	14.5	16.8	8.9	33.9	42.9	15.6	22.1 ± 5.4
16 - 17	0.3	0.5	0.7	0.7	1.3	1.3	0.8 ± 0.2	18.2	13.9	12.6	36.7	30.5	13.0	20.8 ± 4.2
17 - 18	0.3	0.4	0.8	1.9	2.2	1.5	1.2 ± 0.3	12.6	10.1	26.5	31.8	28.3	17.4	21.1 ± 3.7
18 - 19	0.5	0.6	1.0	1.2	0.8	1.8	1.0 ± 0.2	9.9	10.5	33.7	27.4	34.1	17.4	22.2 ± 4.5
19 - 20	0.5	0.6	0.6	0.3	1.3	1.7	0.8 ± 0.2	8.8	11.2	21.1	39.3	36.7	18.8	22.7 ± 5.2

N.D. not determined
* not included in statistical analysis

Appendix 2: Table 31

Experiment 2: Faecal Losses of Haemoglobin Iron (mg/day) in Control and Fluke-infected Sheep

Weeks After Infection	Controls							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - 2	2.00	2.46	1.55	1.66	3.14	2.23	2.17 ± 0.24	1.65	2.03	2.66+	2.48	2.85	1.99	2.28 ± 0.19
4 - 5	1.23	2.06	N.D.	1.44	2.65	1.43	1.76 ± 0.26	1.08	1.72	1.80+	2.40	1.96	2.13	1.86 ± 0.22
5 - 6	0.90	1.79	N.D.	1.12	2.25	1.91	1.59 ± 0.25	0.86	1.90	1.76+	1.70	2.48	1.31	1.65 ± 0.27
6 - 7	1.12	1.57	N.D.	1.12	2.25	1.80	1.57 ± 0.21	0.76	2.61	2.70+	1.63	4.91	3.21	2.62 ± 0.71
7 - 8	1.01	1.12	N.D.	1.01	2.24	1.68	1.41 ± 0.24	2.30	8.69	5.95+	6.51	9.82	2.06	5.88 ± 1.60
8 - 9	0.79	0.90	1.12	1.13	2.02	1.46	1.24 ± 0.18	2.60	15.27	15.84	10.08	16.38	6.05	11.04 ± 2.36
9 - 10	0.80	1.01	0.90	1.46	2.13	1.68	1.33 ± 0.21	1.55	15.10	14.51	10.98	17.86	19.71	13.29 ± 2.65
10 - 11	1.01	0.91	1.11	0.90	2.02	1.91	1.31 ± 0.21	3.05	13.56	11.93	22.52	32.34	19.35	17.13 ± 4.10
11 - 12	0.79	0.90	1.35	1.01	1.91	1.91	1.31 ± 0.20	8.10	13.96	19.54	16.46	26.08	20.10	17.37 ± 2.49
12 - 13	0.89	0.67	1.24	1.03	2.04	1.46	1.22 ± 0.20	9.18	25.64	20.37	19.89	33.24	17.15	20.91 ± 3.30
13 - 14	1.10	1.03	1.01	1.47	1.81	1.56	1.33 ± 0.13	12.76	24.71	18.69	27.25	45.56	21.30	25.05 ± 4.59
14 - 15	0.87	0.87	1.12	1.57	1.78	2.01	1.37 ± 0.20	13.53	29.36	16.09	38.51	51.38	20.46	28.22 ± 5.96
15 - 16	1.02	0.79	0.91	1.35	2.13	1.68	1.31 ± 0.21	15.55	18.09	9.54	35.95	46.27	16.63	23.67 ± 5.80
16 - 17	0.34	0.56	0.78	0.79	1.46	1.46	0.90 ± 0.19	19.84	15.01	13.45	39.12	32.86	13.99	22.38 ± 4.48
17 - 18	0.32	0.46	0.90	2.14	2.47	1.68	1.33 ± 0.37	13.38	10.86	27.82	33.51	30.12	18.53	22.37 ± 3.84
18 - 19	0.56	0.60	1.10	1.32	0.91	2.02	1.09 ± 0.22	10.52	11.09	34.55	28.54	36.14	18.47	23.22 ± 4.67
19 - 20	0.58	0.67	0.65	0.37	1.47	1.91	0.94 ± 0.25	9.28	11.88	21.84	40.61	38.90	19.80	23.72 ± 5.43

N.D. not determined
\+ not included in statistical analysis

Appendix 2: Table 32

Experiment 2: Dietary Iron Intake (mg/day) of Fluke-infected and Control Sheep

Weeks After Infection	Controls							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - 1	372	398	302	277	375	390	352 ± 21	377	399	304	277	387	404	358 ± 22
-1 - 0	372	404	335	288	387	403	365 ± 19	379	404	340	289	388	405	368 ± 18
0 - 1	329	405	322	292	392	403	357 ± 20	329	405	323	292	397	400	358 ± 20
1 - 2	369	403	325	309	403	405	369 ± 17	369	403	325	309	403	405	369 ± 17
2 - 3	363	402	330	290	396	405	364 ± 19	372	405	333	290	398	405	367 ± 19
3 - 4	327	390	287	275	389	405	346 ± 23	327	394	319	275	392	405	352 ± 21
4 - 5	320	401	241	315	391	405	346 ± 27	320	404	312	315	391	405	358 ± 19
5 - 6	310	372	273	308	390	404	343 ± 22	310	404	310	308	391	405	355 ± 20
6 - 7	349	400	242	299	382	405	346 ± 26	349	405	237	299	384	405	347 ± 27
7 - 8	307	396	216	190	384	392	314 ± 38	307	396	216	190	384	392	314 ± 38
8 - 9	246	396	252	236	376	393	317 ± 32	252	396	252	236	376	393	318 ± 32
9 - 10	251	396	270	285	393	396	332 ± 29	251	396	270	285	393	396	332 ± 29
10 - 11	254	393	272	304	396	396	336 ± 27	254	396	272	304	396	396	336 ± 27
11 - 12	287	396	303	307	396	396	348 ± 22	287	396	303	307	396	396	348 ± 22
12 - 13	325	396	284	360	396	396	360 ± 19	325	396	284	360	396	396	360 ± 19
13 - 14	361	396	300	257	396	396	351 ± 24	361	396	300	257	396	396	351 ± 24
14 - 15	363	396	267	341	396	396	360 ± 21	363	396	267	341	396	396	360 ± 21
15 - 16	334	396	281	356	396	396	360 ± 19	334	396	281	356	396	396	360 ± 19
16 - 17	152	265	157	108	228	240	192 ± 25	152	301	175	83	297	266	212 ± 36
17 - 18	198	271	161	143	234	244	209 ± 20	204	299	139	131	237	262	212 ± 27
18 - 19	185	279	168	145	217	231	204 ± 20	193	286	170	133	218	289	215 ± 26
19 - 20	191	286	187	135	215	257	212 ± 22	196	286	220	95	215	311	221 ± 31

APPENDIX 3

Data relevant to Chapter 3:

The Relationship of Host Nutrition and Fascioliasis:

The aetiology of the hypoalbuminaemia which develops in sheep following infection with F. hepatica

Appendix 3. Table 1

Experiment 1: Albumin Pools 6 Weeks Prior to Infection with F. hepatica

Diet	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
Hay Only	Control							
	3	37.9	1.09	72.5	2.09	110.4	3.18	1.91
	6	36.4	1.08	66.0	1.95	102.4	3.03	1.81
	962	35.3	1.03	61.9	1.81	97.2	2.83	1.75
	Mean	36.5	1.07	66.8	1.95	103.3	3.01	1.82
	S.E.	0.8	0.02	3.1	0.08	3.8	0.10	0.05
	Infected							
	989	34.4	1.05	65.4	1.99	99.8	3.04	1.90
	981	33.8	1.08	62.1	1.98	95.9	3.06	1.84
	961	36.4	1.02	66.6	1.86	103.0	2.88	1.83
	Mean	34.9	1.05	64.7	1.94	99.6	2.99	1.86
	S.E.	0.8	0.02	1.3	0.04	2.1	0.06	0.02
	+ 985	32.2	1.00	63.4	1.97	95.6	2.97	1.97
	+ 992	41.1	1.21	74.7	2.20	115.8	3.42	1.82
Hay + Compound Diet	Control							
	7	38.5	1.15	71.3	2.14	103.1	3.09	1.85
	952	39.3	1.14	70.8	2.05	110.1	3.18	1.80
	964	37.6	1.04	73.9	2.04	111.5	3.08	1.97
	Mean	38.5	1.11	72.0	2.08	108.2	3.12	1.87
	S.E.	0.5	0.03	1.0	0.03	2.6	0.03	0.05
	Infected							
	5	38.2	1.14	76.1	2.27	114.3	3.41	1.99
	994	35.8	1.09	62.1	1.89	97.9	2.98	1.74
	965	30.1	0.91	59.2	1.79	89.3	2.70	1.96
	Mean	34.7	1.05	65.8	1.98	100.5	3.03	1.90
	S.E.	2.4	0.07	5.2	0.15	7.3	0.21	0.08
	+ 954	38.4	1.19	74.4	2.32	112.8	3.51	1.94
	+ 4	37.3	1.10	73.4	2.17	110.7	3.27	1.96

+ Infected sheep without pair-fed control partners

Appendix 3. Table 2

Experiment 1: Albumin Pools at the Time of Infection with F. hepatica

Diet	Sheep No.	CA		EA		TA		EA/CA
		g	g/kg	g	g/kg	g	g/kg	
Hay Only	Control							
	3	37.4	1.08	69.5	2.01	106.9	3.09	1.86
	6	28.4	0.93	57.6	1.89	86.0	2.82	2.03
	962	38.7	1.14	63.7	1.88	102.4	3.02	1.65
	Mean	34.8	1.05	63.6	1.93	98.4	2.98	1.85
	S.E.	3.2	0.06	3.4	0.04	6.4	0.08	0.10
	Infected							
	989	34.1	0.99	59.6	1.74	93.7	2.73	1.75
	981	30.9	1.04	55.0	1.85	85.9	2.89	1.78
	961	30.9	0.94	60.0	1.82	90.9	2.76	1.94
	Mean	32.0	0.99	58.2	1.80	90.2	2.79	1.82
	S.E.	1.1	0.03	1.6	0.03	2.3	0.05	0.06
	* 985	34.3	1.06	58.3	1.79	92.6	2.85	1.70
	* 992	42.3	1.20	75.2	2.13	117.5	3.33	1.78
Hay + Compound Diet	Control							
	7	41.2	1.09	76.5	2.02	117.7	3.11	1.86
	952	47.5	1.35	71.3	2.03	118.8	3.38	1.50
	964	40.0	1.10	73.2	2.02	113.2	3.13	1.83
	Mean	42.9	1.18	73.7	2.02	116.6	3.21	1.73
	S.E.	2.3	0.08	1.5	0.00	1.7	0.09	0.12
	Infected							
	5	40.4	1.08	74.3	1.99	114.7	3.07	1.84
	994	44.5	1.21	80.1	2.18	124.6	3.39	1.80
	965	39.5	1.10	69.9	1.95	109.4	3.06	1.77
	Mean	41.5	1.13	74.8	2.04	116.2	3.17	1.80
	S.E.	1.5	0.04	3.0	0.07	4.5	0.11	0.02
	* 954	37.9	1.11	70.4	2.05	108.3	3.16	1.86
	* 4	43.3	1.16	76.6	2.05	119.9	3.21	1.77

* Infected sheep without pair-fed control partners.

Appendix 3. Table 3

Experiment 1: Albumin Pools 7 Weeks After Infection with F. hepatica

Diet	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
Hay Only	Control							
	3	36.6	1.11	63.3	1.91	99.9	3.02	1.73
	6	27.2	0.92	50.2	1.69	77.4	2.61	1.85
	962	32.1	1.00	59.6	1.85	91.7	2.85	1.86
	Mean	32.0	1.01	57.7	1.82	89.7	2.83	1.81
	S.E.	2.7	0.05	3.9	0.07	6.6	0.12	0.04
	Infected							
	989	28.5	0.91	46.5	1.48	75.0	2.38	1.63
	981	27.2	0.95	45.5	1.59	72.7	2.54	1.67
	961	24.8	0.82	43.2	1.43	68.0	2.25	1.74
	Mean	26.8	0.89	45.1	1.50	71.9	2.39	1.68
	S.E.	1.1	0.04	1.0	0.05	2.1	0.08	0.03
	+ 985	20.0	0.71	35.5	1.27	55.5	1.98	1.78
	+ 992	37.8	1.09	67.2	1.93	105.0	3.02	1.78
Hay + Compound Diet	Control							
	7	41.4	1.18	70.8	2.01	112.2	3.19	1.71
	952	49.6	1.38	79.0	2.20	128.6	3.58	1.59
	964	44.8	1.28	81.4	2.33	126.2	3.61	1.82
	Mean	45.3	1.28	77.1	2.18	122.3	3.46	1.71
	S.E.	2.4	0.06	3.2	0.09	5.1	0.14	0.07
	Infected							
	5	34.9	0.99	60.7	1.72	95.6	2.72	1.74
	994	43.8	1.17	72.4	1.94	116.2	3.12	1.65
	965	30.1	0.87	50.4	1.45	80.5	2.32	1.67
	Mean	36.3	1.01	61.2	1.70	97.4	2.72	1.69
	S.E.	4.0	0.09	6.4	0.14	10.3	0.23	0.03
	+ 954	30.7	0.98	57.9	1.84	88.6	2.82	1.89
	+ 4	40.1	1.15	75.7	2.18	115.8	3.33	1.89

+ Infected sheep without pair-fed control partners

Appendix 3. Table 4

Experiment 1: Albumin Pools 10 Weeks After Infection with F. hepatica

Diet	Sheep No.	CA		EA		TA		EA/CA
		g	g/kg	g	g/kg	g	g/kg	
Hay Only	Control							
	3	38.9	1.23	59.6	1.88	98.5	3.11	1.53
	6	28.4	0.93	46.3	1.52	74.7	2.46	1.63
	962	34.1	1.12	55.6	1.83	89.7	2.95	1.63
	Mean	33.8	1.09	53.8	1.74	87.6	2.84	1.60
	S.E.	3.0	0.09	3.9	0.11	6.9	0.20	0.03
	Infected							
	989	20.3	0.70	25.3	0.88	45.6	1.58	1.25
	981	22.7	0.82	31.3	1.13	54.0	1.95	1.38
	961	14.9	0.54	20.1	0.72	35.0	1.26	1.35
	Mean	19.3	0.69	25.6	0.91	44.9	1.60	1.33
	S.E.	2.3	0.08	3.2	0.12	5.5	0.20	0.04
	* 985	16.7	0.59	23.7	0.84	40.4	1.42	1.44
	* 992	30.4	0.90	42.0	1.24	72.4	2.13	1.41
Hay + Compound Diet	Control							
	7	43.6	1.24	71.8	2.03	115.4	3.27	1.65
	952	46.8	1.34	71.5	2.04	118.3	3.38	1.53
	964	46.4	1.33	77.7	2.22	124.1	3.55	1.68
	Mean	45.6	1.30	73.7	2.10	119.3	3.40	1.62
	S.E.	1.0	0.03	2.0	0.06	2.6	0.08	0.05
	Infected							
	5	29.9	0.87	49.3	1.44	79.2	2.32	1.65
	994	38.5	1.08	55.8	1.56	94.3	2.64	1.45
	965	29.1	0.88	45.4	1.38	74.5	2.26	1.56
	Mean	32.5	0.94	50.2	1.46	82.7	2.41	1.55
	S.E.	3.0	0.07	3.0	0.05	6.0	0.12	0.06
	* 954	36.8	1.18	55.6	1.78	92.4	2.95	1.51
	* 4	37.7	1.09	59.2	1.71	96.9	2.80	1.57

* Infected Sheep without pair-fed control partners.

Appendix 3. Table 5

Experiment 1: Albumin Pools 14 Weeks After Infection with F. hepatica

Diet	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
Hay Only	Control							
	3	28.5	1.02	41.0	1.46	69.5	2.48	1.44
	6	22.9	0.83	34.3	1.24	57.2	2.07	1.50
	962	25.6	0.88	40.0	1.38	65.6	2.26	1.56
	Mean	25.7	0.91	38.4	1.36	64.1	2.27	1.50
	S.E.	1.6	0.06	2.1	0.06	3.6	0.12	0.03
	Infected							
	989	10.2	0.38	8.1	0.30	18.3	0.68	0.79
	981	11.1	0.42	10.2	0.39	21.3	0.80	0.92
	961	13.5	0.49	14.0	0.52	27.5	1.01	1.04
	Mean	11.6	0.43	10.8	0.40	22.4	0.83	0.92
	S.E.	1.0	0.03	1.7	0.06	2.7	0.10	0.07
	* 985	10.5	0.43	10.3	0.42	20.8	0.85	0.98
	* 992	22.3	0.66	26.3	0.78	48.5	1.44	1.18
Hay + Compound Diet	Control							
	7	40.2	1.10	60.3	1.65	100.5	2.75	1.50
	952	42.8	1.21	66.8	1.89	109.6	3.10	1.56
	964	40.1	1.15	65.0	1.86	105.1	3.01	1.62
	Mean	41.0	1.15	64.0	1.80	105.1	2.95	1.56
	S.E.	0.9	0.03	1.9	0.08	2.6	0.10	0.03
	Infected							
	5	18.2	0.54	25.7	0.76	44.0	1.30	1.41
	994	22.0	0.60	20.3	0.55	42.3	1.15	0.92
	965	16.4	0.52	22.0	0.70	38.4	1.22	1.34
	Mean	18.9	0.55	22.7	0.67	41.6	1.22	1.22
	S.E.	1.7	0.02	1.6	0.06	1.7	0.04	0.15
	* 954	22.8	0.75	30.4	1.00	53.2	1.74	1.33
	* 4	27.5	0.76	35.2	0.97	62.7	1.73	1.28

* Infected sheep without pair-fed control partners.

Appendix 3. Table 6

Experiment 1: Albumin Pools 17 Weeks after Infection with F. hepatica

Diet	Sheep No.	CA		EA		TA		EA/CA
		g	g/kg	g	g/kg	g	g/kg	
Hay + Compound Diet	Control							
	7	34.9	0.97	48.2	1.34	83.1	2.30	1.37
	952	31.2	0.98	43.1	1.36	74.3	2.34	1.38
	964	32.0	0.90	44.2	1.24	80.0	2.24	1.54
	Mean	32.7	0.95	45.2	1.31	79.1	2.29	1.43
	S.E.	1.1	0.24	1.5	0.04	2.6	0.03	0.05
	Infected							
	5	18.3	0.55	19.8	0.59	38.1	1.14	1.07
	994	6.4	0.17	4.3	0.12	10.7	0.29	0.67
	965	16.7	0.53	16.0	0.51	32.7	1.05	0.96
	Mean	13.8	0.42	13.4	0.41	27.2	0.83	0.90
	S.E.	3.7	0.12	4.7	0.14	8.4	0.27	0.12
	* 954	14.4	0.47	17.6	0.57	32.0	1.03	1.23
	* 4	12.8	0.37	11.4	0.33	24.2	0.69	0.87

* Infected sheep without pair-fed control partners.

Appendix 3. Table 7

Experiment 1: Albumin Pools 20 Weeks After Infection with F. hepatica

Diet	Sheep No.	CA		EA		TA		EA/CA
		g	g/kg	g	g/kg	g	g/kg	
Hay + Compound Diet	Control							
	7	36.8	1.03	N.D.	N.D.	N.D.	N.D.	N.D.
	† 952	-	-	-	-	-	-	-
	964	30.4	0.94	"	"	"	"	"
	Mean	33.6	0.99					
	S.E.	3.2	0.04					
	Infected							
	5	10.1	0.31	"	"	"	"	"
	† 994	-	-	-	-	-	-	-
	965	7.2	0.24	"	"	"	"	"
	Mean	8.7	0.28					
	S.E.	1.5	0.03					
	* 954	11.7	0.39	"	"	"	"	"
	* 4	6.4	0.20	"	"	"	"	"

* Infected sheep without pair-fed control partners.

† Died or killed prior to measurement.

N.D. Not determined.

Appendix 3. Table 8

Experiment 1: Apparent Albumin Half-life (hours) in Control and Fluke-infected Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
Pre	505	583	633	574 ± 37	595	791	544	643 ± 75	511	426
0 - 7	526	602	568	565 ± 22	587	624	538	583 ± 25	496	422
7 - 10	705	726	693	708 ± 10	304	448	383	378 ± 42	384	336
10 - 14	1011	1270	733	1005 ±155	209	322	399	310 ± 55	372	247

* Infected sheep without pair-fed control partners.

Appendix 3. Table 9

Experiment 1: Apparent Albumin Half-life (hours) in Control and Fluke-infected Sheep Fed Hay Plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
Pre	488	579	602	556 ± 35	449	551	491	497 ± 30	562	523
0 - 7	493	468	587	516 ± 36	474	526	476	492 ± 17	531	497
7 - 10	515	532	643	563 ± 40	448	348	452	416 ± 34	485	403
10 - 14	688	595	624	636 ± 27	397	233	378	336 ± 52	338	329
14 - 17	608	636	652	632 ± 13	233	119	288	213 ± 50	192	248
17 - 20	720	Dead	936	828 ± 108	151	Dead	161	156 ± 5	148	182

* Infected sheep without pair-fed control partners.

Appendix 3. Table 10

Experiment 1: Fractional Catabolic Rate of Albumin F(CA) in Control and Fluke-infected Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
Pre	0.057	0.056	0.041	0.051 ± 0.005	0.067	0.032	0.053	0.051 ± 0.010	0.054	0.067
0 - 7	0.060	0.058	0.043	0.054 ± 0.005	0.073	0.047	0.061	0.060 ± 0.008	0.058	0.060
7 - 8	0.047	0.046	0.049	0.047 ± 0.001	0.083	0.061	0.076	0.073 ± 0.006	0.064	0.084
8 - 9	0.044	0.036	0.035	0.038 ± 0.003	0.112	0.061	0.095	0.089 ± 0.015	0.062	0.101
9 - 10	0.037	0.026	0.032	0.031 ± 0.003	0.099	0.063	0.091	0.084 ± 0.010	0.041	0.110
10 - 11	0.031	0.028	0.036	0.032 ± 0.002	0.123	0.082	0.096	0.100 ± 0.012	0.060	0.134
11 - 12	0.037	0.030	0.033	0.033 ± 0.002	0.174	0.091	0.104	0.123 ± 0.026	0.089	0.149
12 - 13	0.035	0.040	0.031	0.035 ± 0.003	0.198	0.097	0.122	0.139 ± 0.030	0.110	0.168
13 - 14	0.032	0.034	0.046	0.037 ± 0.004	0.227	0.115	0.123	0.155 ± 0.036	0.125	0.183

* Infected sheep without pair-fed control partners.

Appendix 3. Table 11

Experiment 1: Fractional Catabolic Rate of Albumin F(CA) in Control and Fluke-infected Sheep Fed Hay plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
Pre	0.077	0.071	0.066	0.071 ± 0.003	0.075	0.061	0.077	0.071 ± 0.005	0.072	0.071
0 - 7	0.081	0.071	0.065	0.072 ± 0.005	0.081	0.078	0.079	0.079 ± 0.001	0.080	0.083
7 - 8	0.077	0.070	0.063	0.070 ± 0.000	0.090	0.077	0.083	0.083 ± 0.004	0.091	0.089
8 - 9	0.070	0.065	0.056	0.064 ± 0.004	0.082	0.102	0.079	0.088 ± 0.000	0.080	0.087
9 - 10	0.061	0.064	0.051	0.059 ± 0.004	0.083	0.110	0.078	0.090 ± 0.010	0.075	0.090
10 - 11	0.066	0.072	0.058	0.065 ± 0.004	0.106	0.123	0.089	0.106 ± 0.010	0.068	0.119
11 - 12	0.062	0.066	0.045	0.058 ± 0.006	0,106	0.172	0.073	0.117 ± 0.029	0.091	0.106
12 - 13	0.066	0.060	0.044	0.057 ± 0.007	0.118	0.164	0.100	0.127 ± 0.019	0.107	0.120
13 - 14	0.065	0.062	0.049	0.059 ± 0.005	0.129	0.175	0.119	0.141 ± 0.017	0.142	0.122
14 - 15	0.067	0.064	0.057	0.063 ± 0.003	0.155	0.193	0.125	0.158 ± 0.020	0.177	0.162
15 - 16	0.061	0.062	0.063	0.062 ± 0.001	0.175	0.241	0.122	0.179 ± 0.034	0.177	0.212
16 - 17	0.049	0.060	0.057	0.055 ± 0.003	0.212	0.220	0.150	0.194 ± 0.022	0.191	0.227
17 - 18	0.051	Dead	0.045	0.048 ± 0.003	0.194	Dead	0.146	0.170 ± 0.024	0.213	0.236
18 - 19	0.047		0.041	0.044 ± 0.003	0.234		0.184	0.209 ± 0.025	0.258	0.321
19 - 20	0.047		0.037	0.042 ± 0.005	0.286		0.252	0.269 ± 0.017	0.273	0.316

* Infected sheep without pair-fed control partners.

Appendix 3. Table 12

Experiment 1: Total Amount of Albumin Catabolised (g/day) by Control and Fluke-infected Sheep fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
Pre	2.13	1.59	1.59	1.77 ± 0.18	2.28	1.27	1.64	1.74 ± 0.29	1.85	2.83
0 - 7	2.20	1.58	1.38	1.72 ± 0.25	2.09	1.28	1.51	1.63 ± 0.24	1.16	2.27
7 - 8	1.74	1.26	1.59	1.53 ± 0.14	2.26	1.61	1.76	1.88 ± 0.20	1.25	3.07
8 - 9	1.66	1.00	1.16	1.27 ± 0.20	2.73	1.53	1.88	2.05 ± 0.36	1.14	3.44
9 - 10	1.42	0.73	1.08	1.08 ± 0.20	2.15	1.49	1.50	1.71 ± 0.22	0.71	3.47
10 - 11	1.17	0.78	1.19	1.05 ± 0.13	2.34	1.75	1.42	1.84 ± 0.27	0.95	3.94
11 - 12	1.30	0.79	1.02	1.04 ± 0.15	2.87	1.67	1.50	2.01 ± 0.43	1.28	4.07
12 - 13	1.13	1.00	0.89	1.01 ± 0.07	2.77	1.50	1.71	1.99 ± 0.39	1.41	4.25
13 - 14	0.95	0.80	1.38	1.04 ± 0.17	2.61	1.44	1.54	1.86 ± 0.37	1.41	4.26

* Infected sheep without pair-fed control partners.

Appendix 3. Table 13

Experiment 1: Total Amount of Albumin Catabolised (g/day) by Control and Fluke-infected Sheep Fed Hay plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
Pre	3.07	3.09	2.56	2.91 ± 0.17	2.95	2.45	2.68	2.69 ± 0.14	2.74	2.86
0 - 7	3.27	3.45	2.82	3.18 ± 0.19	3.17	3.27	2.79	3.08 ± 0.15	2.58	3.19
7 - 8	3.21	3.44	2.84	3.16 ± 0.17	3.07	3.31	2.49	2.96 ± 0.24	2.88	3.53
8 - 9	2.97	3.13	2.55	2.88 ± 0.17	2.66	4.19	2.34	3.06 ± 0.57	2.70	3.38
9 - 10	2.63	3.02	2.36	2.67 ± 0.19	2.55	4.32	2.29	3.05 ± 0.64	2.68	3.43
10 - 11	2.84	3.33	2.64	2.94 ± 0.20	3.01	4.48	2.45	3.31 ± 0.61	2.39	4.33
11 - 12	2.62	2.99	1.98	2.53 ± 0.29	2.70	5.56	1.77	3.34 ± 1.14	2.88	3.59
12 - 13	2.74	2.66	1.87	2.42 ± 0.28	2.66	4.62	2.12	3.13 ± 0.76	3.02	3.77
13 - 14	2.64	2.68	2.00	2.44 ± 0.22	2.53	4.24	2.14	2.97 ± 0.64	3.49	3.51
14 - 15	2.64	2.61	2.21	2.49 ± 0.14	2.82	3.76	2.07	2.88 ± 0.49	3.79	4.08
15 - 16	2.29	2.29	2.27	2.28 ± 0.00	3.19	3.45	2.03	2.89 ± 0.44	3.29	4.28
16 - 17	1.76	1.99	1.90	1.88 ± 0.07	3.88	1.98	2.51	2.79 ± 0.57	3.04	3.45
17 - 18	1.80	Dead	1.43	1.62 ± 0.18	3.30	Dead	2.22	2.76 ± 0.54	2.98	2.76
18 - 19	1.69		1.28	1.49 ± 0.20	3.32		2.21	2.77 ± 0.55	3.38	3.05
19 - 20	1.72		1.13	1.43 ± 0.29	3.29		2.22	2.76 ± 0.53	3.33	2.34

* Infected sheep without pair-fed control partners.

Appendix 3. Table 14

Experiment 1: Faecal Plasma Clearance (ml/day) in Control and Fluke-infected Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
Pre	23.0	17.4	20.9	20.4 ± 1.6	22.9	22.6	30.3	25.3 ± 2.5	26.9	31.1
0 - 7	18.6	24.3	22.4	21.8 ± 1.7	29.2	25.3	31.0	28.5 ± 1.7	24.8	39.8
7 - 8	19.8	21.4	22.8	21.3 ± 0.9	50.8	39.4	42.4	44.2 ± 3.4	32.9	69.9
8 - 9	19.0	24.0	22.0	21.7 ± 1.5	85.4	51.1	78.9	71.8 ± 10.5	31.3	115.0
9 - 10	23.6	22.3	25.3	23.7 ± 0.9	120.0	85.9	81.1	95.7 ± 12.2	39.9	153.9
10 - 11	16.8	19.6	22.3	19.6 ± 1.6	220.6	165.7	130.8	172.4 ± 26.1	77.9	213.9
11 - 12	17.2	16.4	35.9	23.2 ± 6.4	225.4	185.0	151.5	187.3 ± 21.4	119.2	185.3
12 - 13	17.8	24.2	28.7	23.6 ± 3.2	229.5	208.4	167.0	201.6 ± 18.4	138.6	198.8
13 - 14	17.5	24.1	29.3	23.6 ± 3.4	282.2	256.0	213.0	250.4 ± 20.2	210.2	250.5

* Infected Sheep without pair-fed control partners.

Appendix 3. Table 15

Experiment 1: Faecal Plasma Clearance (ml/day) in Control and Fluke-infected Sheep Fed Hay Plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
Pre	29.1	32.8	30.2	30.7 ± 1.1	33.3	31.4	36.8	33.8 ± 1.6	35.8	33.0
0 - 7	28.9	36.2	28.3	31.1 ± 2.5	34.9	35.4	29.1	33.1 ± 2.0	46.8	38.0
7 - 8	27.4	24.2	43.3	31.6 ± 5.9	38.3	60.5	41.1	46.6 ± 7.0	51.8	39.7
8 - 9	29.1	32.3	31.5	31.0 ± 1.0	45.9	86.2	43.6	58.6 ± 13.8	60.2	70.9
9 - 10	31.9	35.5	28.5	32.0 ± 2.0	56.1	131.7	55.2	81.0 ± 24.4	84.3	83.5
10 - 11	26.0	37.4	31.6	31.7 ± 3.3	73.3	185.0	69.1	109.1 ± 38.0	93.5	132.1
11 - 12	25.3	34.2	24.3	27.9 ± 3.1	107.0	210.0	100.1	139.0 ± 35.5	100.0	149.2
12 - 13	31.6	33.3	26.3	30.4 ± 2.1	114.5	232.1	121.4	156.0 ± 38.1	132.1	153.1
13 - 14	34.7	41.4	27.6	34.6 ± 4.0	132.0	283.8	146.5	187.4 ± 48.4	156.0	189.7
14 - 15	26.3	33.9	25.2	28.5 ± 2.7	156.8	370.0	140.3	222.4 ± 74.0	164.4	153.8
15 - 16	22.4	21.9	29.1	24.5 ± 2.3	186.6	467.2	184.3	279.4 ± 93.9	261.7	162.4
16 - 17	18.5	13.6	18.8	17.0 ± 1.7	227.9	432.5	222.3	294.2 ± 69.2	280.6	203.0
17 - 18	26.2	Dead	15.3	20.8 ± 5.5	261.9	Dead	256.7	259.3 ± 2.6	330.5	343.8
18 - 19	28.9		26.1	27.5 ± 1.4	304.7		312.9	308.8 ± 4.1	391.9	416.7
19 - 20	30.6		25.5	28.1 ± 2.6	329.4		384.9	357.2 ± 27.8	437.7	502.9

* Infected sheep without pair-fed control partners.

Appendix 3. Table 16

Experiment 1: The Enteric Albumin Loss (g/day) Experienced by Control and Fluke-infected Sheep Fed Hay Only

Weeks After Infection	Control				Infected					
	3	6	962	Mean ± S.E.	989	981	961	Mean ± S.E.	985*	992*
Pre	0.65	0.40	0.57	0.54 ± 0.07	0.58	0.52	0.70	0.60 ± 0.05	0.65	0.93
0 - 7	0.59	0.48	0.54	0.54 ± 0.03	0.63	0.51	0.62	0.59 ± 0.04	0.40	1.05
7 - 8	0.54	0.51	0.60	0.55 ± 0.03	0.93	0.63	0.57	0.71 ± 0.11	0.29	1.63
8 - 9	0.55	0.52	0.60	0.56 ± 0.02	1.36	0.81	1.01	1.06 ± 0.16	0.30	2.20
9 - 10	0.66	0.49	0.69	0.61 ± 0.06	1.69	1.23	0.74	1.22 ± 0.27	0.42	2.94
10 - 11	0.43	0.40	0.57	0.47 ± 0.05	2.15	1.78	1.09	1.67 ± 0.31	0.54	3.01
11 - 12	0.39	0.32	0.73	0.48 ± 0.13	2.07	1.66	1.09	1.61 ± 0.28	1.16	2.89
12 - 13	0.43	0.46	0.64	0.51 ± 0.07	1.93	1.30	1.29	1.51 ± 0.21	0.85	2.94
13 - 14	0.39	0.44	0.57	0.47 ± 0.05	1.92	1.38	1.73	1.68 ± 0.16	1.58	3.33

* Infected sheep without pair-fed control partners.

Appendix 3. Table 17

Experiment I: The Enteric Albumin Loss (g/day) Experienced by Control and Fluke-infected Sheep Fed Hay Plus Compound Diet

Weeks After Infection	Control				Infected					
	7	952	964	Mean ± S.E.	5	994	965	Mean ± S.E.	954*	4*
Pre	0.90	1.00	0.84	0.91 ± 0.05	0.91	0.95	0.82	0.89 ± 0.04	1.10	0.99
0 - 7	0.85	1.19	0.85	0.96 ± 0.11	0.84	0.99	0.64	0.82 ± 0.10	1.08	0.95
7 - 8	0.81	0.78	1.48	1.02 ± 0.23	0.98	1.50	0.96	1.15 ± 0.18	1.21	1.04
8 - 9	0.91	0.99	1.00	0.97 ± 0.03	0.98	1.75	0.92	1.22 ± 0.27	1.32	1.55
9 - 10	1.08	1.13	0.89	1.03 ± 0.07	1.03	2.86	0.90	1.60 ± 0.63	1.75	1.78
10 - 11	0.87	1.19	0.99	1.02 ± 0.09	1.05	3.44	0.71	1.73 ± 0.86	1.71	2.54
11 - 12	0.75	0.93	0.67	0.78 ± 0.08	1.28	2.96	1.35	1.86 ± 0.55	1.63	2.33
12 - 13	0.94	0.95	0.73	0.87 ± 0.07	1.37	2.34	1.40	1.70 ± 0.32	1.52	2.89
13 - 14	1.00	1.18	0.75	0.98 ± 0.12	1.40	3.26	1.38	2.01 ± 0.60	2.00	2.79
14 - 15	0.80	0.98	0.75	0.84 ± 0.07	1.79	3.07	1.45	2.10 ± 0.49	2.15	2.49
15 - 16	0.57	0.51	0.65	0.58 ± 0.04	2.18	2.66	1.70	2.18 ± 0.28	2.70	1.54
16 - 17	0.47	0.30	0.41	0.39 ± 0.05	2.60	1.56	2.11	2.09 ± 0.30	2.36	1.44
17 - 18	0.64	Dead	0.32	0.48 ± 0.16	1.94	Dead	1.62	1.78 ± 0.16	2.08	1.79
18 - 19	0.73		0.56	0.65 ± 0.08	2.56		1.41	1.99 ± 0.57	3.06	1.75
19 - 20	0.80		0.52	0.66 ± 0.14	2.04		1.69	1.87 ± 0.17	3.11	1.76

* Infected sheep without pair-fed control partners.

Appendix 3: Table 18

Experiment 1: Albumin Synthesis (mg/kg/day) in Sheep infected with *F.hepatica* and worm-free Controls

Diet	Sheep No.	Pre-Infection	Weeks After Infection 0 - 7	7 - 14	14 - 17
Hay Only	Control				
	3	58.6	61.4	22.9	
	6	37.1	47.1	17.1	
	962	50.0	35.7	21.4	
	Mean	48.6	48.1	20.5	
	S.E.	6.2	7.4	1.7	
	Infected				
	989	64.3	51.4	45.7	
	981	34.3	34.3	18.6	
	961	40.0	32.9	27.1	
	Mean	46.2	39.5	30.5	
	S.E.	9.2	5.9	8.0	
	+ 985	55.7	12.9	15.7	
	+ 992	82.9	57.1	77.1	
Hay + Compound Diet	Control				
	7	95.7	85.7	71.4	38.6
	952	94.3	102.9	74.3	18.6
	964	71.4	87.1	54.3	27.1
	Mean	87.1	91.9	66.7	28.1
	S.E.	7.9	5.5	6.2	5.8
	Infected				
	5	84.3	77.1	48.6	90.0
	994	88.6	84.3	77.1	41.4
	965	94.0	62.9	41.4	61.4
	Mean	89.0	74.8	55.7	64.3
	S.E.	2.8	6.3	10.9	14.1
	+ 954	78.6	67.1	68.6	77.1
	+ 4	85.7	85.7	71.4	60.0

+ Infected sheep without pair-fed control partners

Appendix 3. Table 19

Experiment 2: Albumin Pools 2 Weeks Prior to Infection with *F. hepatica*

Sheep No.	CA		EA		TA		EA/CA
	g	g/kg	g	g/kg	g	g/kg	
Control							
70	37.4	1.49	56.5	2.24	93.9	3.73	1.51
51	41.0	1.55	71.7	2.71	112.7	4.25	1.75
80	33.4	1.49	55.4	2.47	88.8	3.96	1.66
98	32.6	1.38	51.5	2.18	84.1	3.56	1.58
83	41.7	1.62	69.7	2.71	111.4	4.44	1.67
97	43.1	1.56	70.0	2.53	113.1	4.08	1.62
Mean	38.2	1.52	62.5	2.47	100.7	4.00	1.63
S.E.	1.8	0.03	3.7	0.09	5.4	0.13	0.03
Infected							
78	40.0	1.51	74.4	2.81	114.4	4.32	1.86
77	44.2	1.55	72.4	2.53	116.6	4.08	1.64
60	31.9	1.51	51.8	2.46	83.7	3.97	1.62
68	35.6	1.61	49.9	2.26	85.5	3.87	1.40
54	37.2	1.45	67.4	2.63	104.6	4.09	1.81
61	41.8	1.51	67.7	2.44	109.5	3.95	1.62
Mean	38.5	1.52	63.9	2.52	102.4	4.05	1.66
S.E.	1.8	0.02	4.3	0.08	5.9	0.06	0.07

Appendix 3. Table 20

Experiment 2: Albumin Pools 4 Weeks After Infection with F. hepatica

Sheep No.	CA		EA		TA		EA/CA
	g	g/kg	g	g/kg	g	g/kg	
Control							
70	48.0	1.72	66.5	2.38	114.5	4.10	1.39
51	52.3	1.73	80.6	2.67	132.9	4.40	1.54
80	37.9	1.46	69.9	2.64	107.8	4.07	1.84
98	39.8	1.43	56.6	2.04	96.4	3.47	1.42
83	47.5	1.59	77.7	2.60	125.2	4.19	1.64
97	51.5	1.65	66.2	2.13	117.7	3.78	1.29
Mean	46.2	1.60	69.6	2.41	115.8	4.00	1.52
S.E.	2.4	0.05	3.5	0.11	5.2	0.13	0.08
Infected							
78	42.4	1.44	76.4	2.61	118.8	4.05	1.80
77	54.9	1.62	75.2	2.22	130.1	3.85	1.37
60	31.0	1.28	50.1	2.06	91.1	3.75	1.62
68	34.3	1.43	59.9	2.50	94.2	3.93	1.75
54	49.2	1.63	68.4	2.26	117.6	3.89	1.39
61	49.5	1.59	74.2	2.39	123.7	3.98	1.50
Mean	43.6	1.50	67.4	2.34	112.6	3.91	1.57
S.E.	3.8	0.06	4.3	0.08	6.6	0.04	0.07

Appendix 3. Table 21

Experiment 2: Albumin Pools 8 Weeks After Infection with F. hepatica

Sheep No.	CA		EA		TA		EA/CA
	g	g/kg	g	g/kg	g	g/kg	
Control							
70	47.2	1.56	66.4	2.19	113.6	3.75	1.41
51	55.7	1.69	86.7	2.64	142.4	4.33	1.56
80	38.0	1.68	59.7	2.63	97.7	4.30	1.57
98	40.5	1.42	53.7	1.88	94.2	3.29	1.33
83	52.5	1.63	78.0	2.42	130.5	4.05	1.49
97	50.0	1.51	69.8	2.11	119.8	3.62	1.40
Mean	47.2	1.58	69.1	2.31	116.4	3.89	1.46
S.E.	2.8	0.04	4.9	0.12	7.6	0.17	0.04
Infected							
78	41.1	1.36	60.0	1.99	101.1	3.35	1.46
77	60.0	1.71	80.4	2.29	140.4	4.00	1.34
60	35.3	1.34	57.3	2.17	92.6	3.51	1.62
68	36.6	1.45	47.7	1.89	84.3	3.35	1.30
54	43.5	1.31	55.5	1.68	99.0	2.99	1.28
61	52.6	1.58	71.4	2.14	124.0	3.72	1.36
Mean	44.7	1.46	62.1	2.03	106.9	3.49	1.39
S.E.	3.9	0.06	4.8	0.09	8.6	0.14	0.05

Appendix 3. Table 22

Experiment 2: Albumin pools 12 weeks after Infection with F. hepatica

Sheep No	CA		EA		TA		EA/CA
	g	g/kg	g	g/kg	g	g/kg	
Control							
70	48.7	1.55	64.6	2.06	113.3	3.61	1.33
51	56.2	1.61	78.6	2.25	134.8	3.86	1.40
80	39.7	1.52	62.5	2.40	102.2	3.92	1.57
98	42.8	1.44	59.4	2.00	102.2	3.44	1.39
83	56.9	1.62	83.5	2.38	140.4	4.00	1.47
97	52.4	1.46	73.6	2.06	126.0	3.52	1.41
Mean	49.4	1.53	70.4	2.19	119.8	3.73	1.43
S.E.	2.9	0.03	3.9	0.07	6.7	0.09	0.03
Infected							
78	40.2	1.27	56.2	1.78	96.4	3.05	1.40
77	60.9	1.61	65.7	1.73	126.6	3.34	1.08
60	36.3	1.22	46.8	1.57	83.1	2.79	1.29
68	31.1	1.12	46.1	1.65	96.1	3.44	1.48
54	43.7	1.20	63.4	1.74	107.1	2.93	1.45
61	47.3	1.30	61.2	1.69	108.5	2.99	1.29
Mean	42.9	1.29	56.6	1.69	103.0	3.09	1.33
S.E.	4.2	0.07	3.4	0.03	6.0	0.10	0.06

Appendix 3. Table 23

Experiment 2: Albumin Pools 16 Weeks After Infection with F. hepatica

Sheep No.	CA		EA		TA		EA/CA
	g	g/kg	g	g/kg	g	g/kg	
Control							
70	49.0	1.44	64.5	1.90	113.5	3.34	1.32
51	53.3	1.46	71.6	1.96	124.9	3.42	1.34
80	39.1	1.35	64.7	2.23	103.8	3.58	1.66
98	45.4	1.37	60.8	1.84	106.2	3.21	1.34
83	54.7	1.48	80.9	2.19	135.6	3.67	1.48
97	54.0	1.44	76.5	2.04	130.5	3.47	1.42
Mean	49.3	1.43	69.8	2.03	119.1	3.45	1.43
S.E.	2.5	0.02	3.2	0.06	5.4	0.07	0.05
Infected							
78	37.9	1.12	46.1	1.36	84.0	2.49	1.22
77	62.2	1.59	64.8	1.66	127.0	3.25	1.04
60	35.9	1.19	37.5	1.24	73.4	2.43	1.05
68	20.3	0.67	17.1	0.56	37.4	1.23	0.84
54	39.2	1.00	55.2	1.41	94.4	2.41	1.41
61	37.8	1.05	50.8	1.41	88.6	2.45	1.34
Mean	38.5	1.10	45.3	1.27	84.1	2.38	1.15
S.E.	5.5	0.12	6.7	0.15	11.9	0.26	0.09

Appendix 3. Table 24

Experiment 2: Albumin Pools 20 Weeks After Infection with F. hepatica

Sheep No.	CA		EA		TA		EA/CA
	g	g/kg	g	g/kg	g	g/kg	
Control							
70	44.5	1.43	N.D.	N.D.	N.D.	N.D.	N.D.
51	46.2	1.23	"	"	"	"	"
80	38.2	1.33	"	"	"	"	"
98	36.1	1.19	"	"	"	"	"
83	47.7	1.31	"	"	"	"	"
97	42.1	1.06	"	"	"	"	"
Mean	42.5	1.26					
S.E.	1.9	0.05					
Infected							
78	26.0	0.78	"	"	"	"	"
77	38.2	0.98	"	"	"	"	"
60	29.2	0.96	"	"	"	"	"
68	10.4	0.44	"	"	"	"	"
54	20.6	0.54	"	"	"	"	"
61	31.6	0.84	"	"	"	"	"
Mean	26.0	0.78					
S.E.	3.9	0.09					

N.D. = Not Determined

Appendix 3. Table 25

Experiment 2: Apparent Albumin Half-life (hours) in Control and Fluke-infected Sheep

Sheep No.	Weeks Post-infection				
	-2 - 2	4 - 8	8 - 12	12 - 16	16 - 20
Control					
70	407	549	560	466	715
51	383	457	403	472	647
80	412	N.D.	384	479	912
98	417	558	451	479	1435
83	377	443	414	501	780
97	402	478	403	455	747
Mean	400	497	436	475	873
S.E.	7	24	26	6	118
Infected					
78	378	485	395	343	453
77	348	391	343	306	397
60	351	426*	333	300	223
68	369	410	286	173	132
54	369	396	307	244	238
61	361	461	327	307	291
Mean	363	428	332	279	289
S.E.	5	15	15	25	48

N.D. not determined

* not included in statistical analysis

Appendix 3. Table 26

Experiment 2: Fractional Catabolic Rate of Albumin F(CA) in Control and Fluke-infected Sheep

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 -2	0.079	0.098	0.074	0.071	0.077	0.078	0.080 ± 0.004	0.087	0.094	0.086	0.068	0.084	0.087	0.084 ± 0.004
4 - 5	0.073	0.090	N.D.	0.069	0.078	0.074	0.077 ± 0.004	0.091	0.085	0.093*	0.094	0.081	0.078	0.086 ± 0.003
5 - 6	0.063	0.081	N.D.	0.057	0.076	0.067	0.069 ± 0.004	0.077	0.091	0.088*	0.095	0.076	0.071	0.082 ± 0.005
6 - 7	0.059	0.082	N.D.	0.054	0.073	0.066	0.067 ± 0.005	0.082	0.084	0.086*	0.097	0.082	0.065	0.083 ± 0.005
7 - 8	0.059	0.079	N.D.	0.048	0.074	0.064	0.065 ± 0.005	0.081	0.104	0.082*	0.102	0.093	0.073	0.091 ± 0.006
8 - 9	0.060	0.074	0.078	0.057	0.077	0.078	0.071 ± 0.004	0.081	0.104	0.110	0.122	0.110	0.088	0.103 ± 0.006
9 - 10	0.061	0.073	0.075	0.055	0.069	0.074	0.068 ± 0.003	0.081	0.102	0.104	0.117	0.128	0.099	0.105 ± 0.007
10 - 11	0.054	0.073	0.086	0.055	0.067	0.068	0.067 ± 0.005	0.081	0.099	0.098	0.141	0.125	0.097	0.107 ± 0.009
11 - 12	0.055	0.081	0.079	0.056	0.066	0.069	0.068 ± 0.004	0.091	0.106	0.125	0.142	0.120	0.102	0.114 ± 0.007
12 - 13	0.074	0.072	0.080	0.073	0.078	0.083	0.077 ± 0.002	0.103	0.125	0.105	0.161	0.136	0.124	0.126 ± 0.009
13 - 14	0.066	0.068	0.069	0.064	0.069	0.077	0.069 ± 0.002	0.107	0.109	0.118	0.188	0.155	0.130	0.135 ± 0.012
14 - 15	0.063	0.067	0.072	0.062	0.065	0.072	0.067 ± 0.002	0.105	0.110	0.108	0.222	0.168	0.107	0.137 ± 0.020
15 - 16	0.064	0.068	0.073	0.063	0.061	0.070	0.067 ± 0.002	0.104	0.108	0.105	0.234	0.167	0.115	0.139 ± 0.021
16 - 17	0.045	0.051	0.058	0.041	0.066	0.061	0.054 ± 0.004	0.106	0.097	0,103	0.137	0.146	0.094	0.114 ± 0.009
17 - 18	0.047	0.049	0.039	0.034	0.055	0.054	0.046 ± 0.003	0.098	0.087	0.144	0.326	0.158	0.108	0.154 ± 0.036
18 - 19	0.042	0.039	0.036	0.027	0.044	0.037	0.038 ± 0.002	0.087	0.086	0.236	0.319	0.148	0.110	0.164 ± 0.038
19 - 20	0.046	0.037	0.036	0.031	0.040	0.032	0.037 ± 0.002	0.087	0.083	0.161	0.365	0.146	0.108	0.158 ± 0.043

N.D. not determined
* not included in statistical analysis

Appendix 3. Table 27

Experiment 2: Total Amount of Albumin Catabolised (g/day) by Control and Fluke-infected Sheep

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - 2	3.25	4.37	2.58	2.48	3.36	3.54	3.26 ± 0.28	3.53	4.45	2.73	2.38	3.46	3.86	3.40 ± 0.31
4 - 5	3.50	4.73	N.D.	2.75	3.75	3.80	3.71 ± 0.32	3.84	4.72	2.93*	3.24	3.93	3.88	3.92 ± 0.24
5 - 6	3.01	4.33	N.D.	2.28	3.74	3.42	3.36 ± 0.34	3.23	5.17	2.87*	3.33	3.57	3.59	3.78 ± 0.35
6 - 7	2.80	4.45	N.D.	2.17	3.69	3.33	3.29 ± 0.39	3.41	4.88	2.89*	3.46	3.74	3.34	3.77 ± 0.29
7 - 8	2.79	4.36	N.D.	1.94	3.83	3.22	3.23 ± 0.42	3.35	6.17	2.85*	3.70	4.11	3.81	4.23 ± 0.50
8 - 9	2.85	4.11	2.96	2.33	4.08	3.92	3.38 ± 0.31	3.32	6.25	3.89	4.38	4.79	4.57	4.53 ± 0.41
9 - 10	2.91	4.07	2.89	2.28	3.74	3.76	3.28 ± 0.28	3.30	6.15	3.71	4.04	5.57	5.01	4.63 ± 0.46
10 - 11	2.61	4.09	3.35	2.31	3.71	3.50	3.26 ± 0.28	3.28	6.00	3.52	4.67	5.45	4.78	4.62 ± 0.43
11 - 12	2.67	4.55	3.12	2.38	3.73	3.59	3.34 ± 0.32	3.67	6.44	4.53	4.52	5.23	4.90	4.88 ± 0.38
12 - 13	3.61	4.02	3.16	3.16	4.43	4.37	3.79 ± 0.23	4.11	7.64	3.81	4.78	5.86	5.70	5.32 ± 0.57
13 - 14	3.23	3.75	2.72	2.80	3.88	4.08	3.41 ± 0.24	4.21	6.69	4.27	5.08	6.51	5.68	5.41 ± 0.44
14 - 15	3.08	3.64	2.83	2.76	3.62	3.85	3.30 ± 0.19	4.07	6.79	3.90	5.39	6.85	4.43	5.24 ± 0.54
15 - 16	3.14	3.64	2.86	2.84	3.36	3.77	3.27 ± 0.16	3.97	6.70	3.78	5.05	6.63	4.49	5.10 ± 0.53
16 - 17	2.18	2.67	2.26	1.82	3.56	3.20	2.62 ± 0.27	3.87	5.73	3.62	2.60	5.37	3.49	4.11 ± 0.49
17 - 18	2.23	2.47	1.51	1.43	2.86	2.67	2.20 ± 0.24	3.28	4.63	4.81	5.41	5.09	3.83	4.51 ± 0.33
18 - 19	1.95	1.90	1.39	1.07	2.22	1.72	1.71 ± 0.17	2.65	4.06	7.48	4.50	4.07	3.73	4.42 ± 0.66
19 - 20	2.07	1.74	1.38	1.12	1.95	1.39	1.61 ± 0.15	2.39	3.42	4.83	4.23	3.34	3.50	3.62 ± 0.34

N.D. not determined.
* not included in statistical analysis

Appendix 3. Table 28

Experiment 2: Faecal Plasma Clearance (ml/day) in Control and Fluke-infected Sheep

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - 2	44.7	53.2	45.7	42.0	46.4	56.8	48.1 ± 2.3	46.5	54.9	48.2	27.3	44.9	56.2	46.3 ± 4.2
4 - 5	34.4	41.0	N.D.	39.1	45.2	42.6	40.5 ± 1.8	36.2	49.3	47.5*	25.0	42.1	48.9	40.3 ± 4.5
5 - 6	34.1	40.7	N.D.	29.3	58.9	37.7	40.1 ± 5.1	37.5	45.4	41.3*	28.5	49.9	43.6	41.0 ± 3.7
6 - 7	39.8	44.0	N.D.	30.6	38.0	43.1	39.1 ± 2.4	44.4	54.1	45.8*	39.2	53.6	55.5	49.1 ± 3.2
7 - 8	40.5	47.1	N.D.	37.7	57.2	52.9	47.1 ± 3.7	49.5	72.1	60.7*	59.2	84.1	58.1	64.6 ± 6.1
8 - 9	30.1	35.7	32.3	22.7	43.6	39.9	34.1 ± 3.0	42.5	83.8	78.4	64.1	96.3	64.1	71.5 ± 7.7
9 - 10	39.0	48.4	54.6	25.9	74.3	69.2	51.9 ± 7.4	49.3	105.9	105.9	100.3	143.5	143.5	108.1 ± 14.1
10 - 11	34.5	50.9	58.1	30.3	58.3	50.3	47.1 ± 4.9	54.7	113.5	104.9	157.0	177.6	130.6	123.1 ± 17.6
11 - 12	36.0	73.7	65.5	33.2	54.2	64.6	54.5 ± 6.8	78.4	132.4	140.5	150.3	165.3	152.2	136.5 ± 12.5
12 - 13	26.2	39.8	37.4	31.7	38.8	48.8	37.1 ± 3.1	74.4	132.6	114.8	164.9	170.8	124.3	130.3 ± 14.4
13 - 14	36.1	52.0	41.4	35.5	43.3	61.1	44.9 ± 4.1	96.1	151.3	120.9	198.3	211.2	152.3	155.0 ± 18.0
14 - 15	28.6	36.5	40.3	30,4	35.8	39.1	35.1 ± 1.9	87.4	133.4	108.4	220.1	216.0	128.2	148.9 ± 22.8
15 - 16	30.4	44.4	48.1	27.7	45.9	48.8	40.9 ± 3.8	96.8	118.8	98.6	226.3	233.1	134.5	151.4 ± 25.4
16 - 17	23.2	31.3	26.8	23.4	34.6	33.5	28.8 ± 2.1	95.9	98.7	92.3	224.5	174.8	106.5	132.1 ± 22.4
17 - 18	21.9	44.3	16.2	21.8	24.6	22.8	25.3 ± 4.0	87.1	98.0	155.1	220.2	165.9	115.2	140.3 ± 20.4
18 - 19	27.3	36.4	14.6	19.3	24.4	20.6	23.8 ± 3.1	85.0	106.2	223.8	216.1	195.7	117.3	157.4 ± 25.0
19 - 20	27.4	42.4	15.9	20.6	28.0	22.0	26.1 ± 3.8	82.9	104.8	144.9	289.9	209.7	129.0	160.2 ± 31.4

N.D. not determined
* not included in statistical analysis

Appendix 3. Table 29

Experiment 2: The Enteric Albumin Loss (g/day) Experienced by Control and Fluke-infected Sheep

Weeks After Infection	Control							Infected						
	70	51	80	98	83	97	Mean ± S.E.	78	77	60	68	54	61	Mean ± S.E.
-2 - 2	1.53	1.79	1.47	1.40	1.50	1.85	1.59 ± 0.07	1.58	1.90	1.42	0.90	1.52	2.04	1.56 ± 0.16
4 - 5	1.24	1.51	N.D.	1.11	1.74	1.47	1.41 ± 0.11	1.13	1.83	1.44*	0.79	1.41	1.64	1.36 ± 0.18
5 - 6	1.14	1.46	N.D.	0.89	2.08	1.28	1.37 ± 0.20	1.13	1.69	1.26*	0.88	1.51	1.45	1.33 ± 0.14
6 - 7	1.36	1.56	N.D.	0.94	1.33	1.46	1.33 ± 0.11	1.35	1.95	1.42*	1.24	1.60	1.86	1.60 ± 0.14
7 - 8	1.34	1.71	N.D.	1.17	2.07	1.75	1.61 ± 0.16	1.47	2.49	1.74*	1.79	2.23	1.91	1.98 ± 0.18
8 - 9	1.03	1.30	1.13	0.69	1.59	1.31	1.18 ± 0.12	1.23	2.89	2.31	1.77	2.37	2.14	2.12 ± 0.23
9 - 10	1.37	1.80	1.95	0.78	2.78	2.32	1.83 ± 0.29	1.48	3.68	2.83	2.50	3.52	4.66	3.11 ± 0.45
10 - 11	1.20	1.88	2.03	0.93	2.23	1.68	1.66 ± 0.20	1.57	4.05	2.75	3.77	4.48	3.92	3.42 ± 0.44
11 - 12	1.26	2.59	2.24	1.05	1.98	2.21	1.89 ± 0.25	2.12	4.59	3.75	3.35	4.07	4.48	3.73 ± 0.37
12 - 13	0.91	1.43	1.28	0.94	1.44	1.68	1.28 ± 0.12	1.99	4.75	3.02	3.68	4.32	3.36	3.52 ± 0.40
13 - 14	1.32	1.86	1.42	1.08	1.66	2.11	1.58 ± 0.15	2.57	5.25	3.33	4.42	5.58	4.14	4.22 ± 0.46
14 - 15	1.03	1.32	1.32	0.90	1.33	1.34	1.21 ± 0.08	2.32	4.62	2.93	3.94	5.03	4.26	3.85 ± 0.42
15 - 16	1.06	1.62	1.60	0.83	1.70	1.72	1.42 ± 0.15	2.51	4.06	2.64	3.40	5.11	3.01	3.46 ± 0.40
16 - 17	0.79	1.10	0.92	0.67	1.26	1.12	0.98 ± 0.09	2.23	2.92	2.45	3.10	3.81	2.66	2.86 ± 0.23
17 - 18	0.76	1.52	0.56	0.67	0.88	0.77	0.86 ± 0.14	1.83	2.79	4.19	2.64	3.42	2.40	2.88 ± 0.34
18 - 19	0.93	1.17	0.50	0.61	0.87	0.68	0.79 ± 0.10	1.79	2.67	4.81	2.16	3.58	2.37	2.90 ± 0.46
19 - 20	0.90	1.38	0.53	0.61	1.02	0.68	0.85 ± 0.13	1.60	2.43	3.14	2.23	2.58	2.50	2.41 ± 0.20

N.D. not determined

* not included in statistical analysis

Appendix 3. Table 30

Experiment 2: Albumin Synthesis (mg/kg/day) in Control and Fluke-infected Sheep

Sheep No.	-2 to 8 Weeks After Infection	8 to 16 Weeks After Infection
Control		
70	122.9	95.7
51	160.0	105.7
80	N.D.	118.6
98	91.4	95.7
83	128.6	111.4
97	115.7	112.9
Mean	123.7	106.7
S.E.	11.1	3.9
Infected		
78	112.9	108.6
77	152.9	167.1
60	121.4 +	120.0
68	120.0	140.0
54	118.6	158.6
61	127.1	118.6
Mean	126.3	135.5
S.E.	7.0	9.7

N.D. Not Determined

\+ Not included in statistical analysis

APPENDIX 4

Data relevant to Chapter 4:

The Relationship of Host nutrition and Fascioliasis: The aetiology of the body weight changes which develop in sheep following infection with F. hepatica

Appendix 4. Table 1

Experiment 1: Nitrogen Intake (g/day) of Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 -7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	8.15	7.86	6.66	4.92	
	6	7.21	7.12	8.52	4.69	
	962	8.13	7.56	6.34	5.62	
	Mean	7.83	7.51	7.17	5.08	
	S.E.	0.31	0.21	0.68	0.28	
	Infected					
	989	6.54	7.80	7.07	4.62	
	981	5.23	7.21	8.89	4.33	
	961	6.15	7.59	6.73	5.51	
	Mean	5.97	7.53	7.56	4.82	
	S.E.	0.39	0.17	0.67	0.36	
	+ 985	6.19	7.17	7.31	5.78	
	+ 992	6.90	9.63	10.01	7.12	
Hay + Compound Diet	Control					
	7	15.50	16.53	15.81	14.98	16.63
	952	16.46	18.63	16.98	15.70	
	964	16.43	16.37	15.25	10.26	12.71
	Mean	16.13	17.18	16.01	13.65	14.67
	S.E.	0.32	0.73	0.51	1.71	1.96
	Infected					
	5	14.45	16.64	15.65	15.03	16.17
	994	14.01	18.59	17.18	15.73	
	965	14.90	16.35	15.53	10.38	12.06
	Mean	14.45	17.19	16.12	13.71	14.12
	S.E.	0.26	0.70	0.53	1.68	2.06
	+ 954	14.84	16.41	14.89	10.45	15.79
	+ 4	13.05	16.18	14.71	15.92	12.78

+ Infected sheep without pair-fed control partners

Appendix 4: Table 2

Experiment 1: Faecal Nitrogen Excretion (g/day) of Fluke-infected and Control Sheep.

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	4.92	4.48	3.27	2.86	
	6	4.51	3.75	4.08	2.64	
	962	5.14	4.40	3.44	3.54	
	Mean	4.86	4.21	3.60	3.01	
	S.E.	0.18	0.23	0.25	0.27	
	Infected					
	989	3.92	4.78	3.36	3.29	
	981	3.20	4.21	4.53	2.95	
	961	3.65	4.30	3.23	3.43	
	Mean	3.59	4.43	3.71	3.22	
	S.E.	0.21	0.18	0.41	0.14	
	* 985	3.31	4.28	3.59	3.82	
	* 992	4.46	6.15	4.91	4.90	
Hay + Compound Diet	Control					
	7	6.31	6.18	5.33	5.59	6.25
	952	6.67	8.08	6.42	6.35	
	964	6.31	6.06	5.02	4.06	4.48
	Mean	6.43	6.77	5.59	5.33	5.37
	S.E.	0.12	0.65	0.42	0.67	0.88
	Infected					
	5	5.69	6.38	5.36	5.33	5.99
	994	6.16	7.53	5.69	6.20	
	965	5.86	6.02	5.17	4.29	5.21
	Mean	5.90	6.64	5.41	5.27	5.60
	S.E.	0.14	0.46	0.15	0.55	0.39
	* 954	5.80	5.98	4.89	4.69	5.07
	* 4	4.12	5.71	5.39	6.86	4.05

* Infected sheep without pair-fed control partners.

Appendix 4: Table 3

Experiment 1: Urinary Nitrogen Excretion (g/day) of Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	1.55	2.07	3.92	1.98	
	6	1.73	1.26	3.43	1.22	
	962	1.45	1.51	2.72	1.58	
	Mean	1.58	1.61	3.36	1.59	
	S.E.	0.08	0.24	0.35	0.22	
	Infected					
	989	1.68	1.71	4.66	3.08	
	981	0.96	1.70	4.45	3.01	
	961	2.16	2.53	4.99	3.08	
	Mean	1.60	1.98	4.70	3.06	
	S.E.	0.35	0.28	0.16	0.02	
	* 985	1.62	3.25	6.59	3.53	
	* 992	1.36	2.47	5.76	2.46	
Hay + Compound Diet	Control					
	7	6.48	8.45	10.91	7.74	7.40
	952	7.55	8.35	11.94	8.64	
	964	6.78	7.66	9.91	6.01	7.12
	Mean	6.94	8.15	10.92	7.46	7.26
	S.E.	0.32	0.25	0.59	0.77	0.14
	Infected					
	5	7.15	8.36	11.83	8.47	9.00
	994	6.08	7.50	11.92	11.12	
	965	6.91	7.82	11.39	5.93	7.76
	Mean	6.71	7.89	11.71	8.51	8.38
	S.E.	0.32	0.25	0.16	1.50	0.62
	* 954	6.77	7.43	9.94	4.81	9.48
	* 4	7.52	7.12	10.55	7.10	11.08

* Infected sheep without pair-fed control partners.

Appendix 4: Table 4

Experiment 1: Nitrogen Balance (g/day) of Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	+ 1.68	+ 1.31	- 0.53	+ 0.08	
	6	+ 0.97	+ 2.11	+ 1.01	+ 0.83	
	962	+ 1.54	+ 1.65	+ 0.18	+ 0.50	
	Mean	+ 1.40	+ 1.69	+ 0.22	+ 0.47	
	S.E.	0.22	0.23	0.44	0.22	
	Infected					
	989	+ 0.94	+ 1.31	- 0.95	- 1.75	
	981	+ 1.07	+ 1.30	- 0.09	- 1.63	
	961	+ 0.34	+ 0.76	- 1.49	- 1.00	
	Mean	+ 0.78	+ 1.12	- 0.84	- 1.46	
	S.E.	0.22	0.18	0.41	0.23	
	* 985	+ 1.26	- 0.36	- 2.87	- 1.57	
	* 992	+ 1.08	+ 1.01	- 0.66	- 0.24	
Hay + Compound Diet	Control					
	7	+ 2.71	+ 1.90	- 0.43	+ 1.65	+ 2.98
	952	+ 2.24	+ 2.20	- 1.38	+ 0.72	
	964	+ 3.34	+ 2.65	+ 0.32	+ 0.19	+ 1.11
	Mean	+ 2.76	+ 2.25	- 0.50	+ 0.85	+ 2.05
	S.E.	0.32	0.22	0.49	0.43	0.93
	Infected					
	5	+ 1.61	+ 1.90	- 1.54	+ 1.23	+ 1.18
	994	+ 1.77	+ 3.56	- 0.43	- 1.59	
	965	+ 2.13	+ 2.51	- 1.03	+ 0.16	- 0.91
	Mean	+ 1.84	+ 2.66	- 1.00	- 0.07	+ 0.14
	S.E.	0.15	0.48	0.32	0.82	1.04
	* 954	+ 2.27	+ 3.00	+ 0.06	+ 0.95	+ 1.24
	* 4	+ 1.41	+ 3.35	- 1.23	+ 1.96	- 2.35

* Infected sheep without pair-fed control partners.

Appendix 4: Table 5

Experiment 1: Apparent Dry Matter Digestibility Coefficients (%) in Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	61.2	61.3	62.1	64.9	
	6	63.3	65.4	64.8	67.2	
	962	63.6	64.8	60.1	65.5	
	Mean	62.7	63.8	62.3	65.9	
	S.E.	1.3	2.2	2.4	1.2	
	Infected					
	989	61.5	62.4	63.0	62.4	
	981	72.2	65.2	61.8	65.6	
	961	60.8	61.4	64.8	65.0	
	Mean	64.8	63.0	63.2	64.3	
	S.E.	6.4	2.0	1.5	1.7	
	* 985	67.1	64.2	61.9	62.8	
	* 992	61.6	61.2	63.5	61.1	
Hay + Compound Diet	Control					
	7	61.4	62.1	62.2	64.2	59.6
	952	61.8	59.8	60.0	61.5	
	964	64.4	62.7	64.6	65.3	62.0
	Mean	62.5	61.5	62.3	63.7	60.8
	S.E.	1.6	1.5	2.3	2.0	1.7
	Infected					
	5	63.3	61.6	59.1	65.7	58.7
	994	61.6	62.4	64.4	62.3	
	965	62.6	63.1	62.9	65.8	57.9
	Mean	62.5	62.4	62.1	64.6	58.3
	S.E.	0.9	0.8	2.7	2.0	0.6
	* 954	61.1	60.6	63.3	62.7	58.3
	* 4	65.9	63.5	62.5	61.9	59.0

* Infected sheep without pair-fed control partners.

Appendix 4: Table 6

Experiment 1: Apparent Organic Matter Digestibility Coefficients (%) in Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection			
			5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	63.1	63.0	63.5	65.5	
	6	62.8	67.2	65.7	67.9	
	962	65.7	66.6	61.1	66.3	
	Mean	63.9	65.6	63.4	66.6	
	S.E.	1.6	2.3	2.3	1.2	
	Infected					
	989	63.1	64.0	64.0	63.5	
	981	74.2	67.0	63.0	66.8	
	961	63.3	63.1	66.3	66.0	
	Mean	66.9	64.7	64.4	65.4	
	S.E.	6.4	2.0	1.7	1.7	
	* 985	69.4	66.5	63.2	63.5	
	* 992	64.0	62.7	63.9	62.0	
Hay + Compound Diet	Control					
	7	63.8	64.1	64.0	65.9	61.2
	952	63.4	61.0	61.1	62.4	
	964	65.2	63.6	65.6	66.1	62.6
	Mean	64.1	62.9	63.6	64.8	61.9
	S.E.	0.9	1.7	2.3	2.1	1.0
	Infected					
	5	64.9	62.9	60.2	66.8	60.5
	994	63.2	64.1	65.9	63.9	
	965	64.1	64.3	64.5	66.9	59.5
	Mean	64.1	63.8	63.5	65.9	60.0
	S.E.	0.9	0.8	3.0	1.7	0.7
	* 954	63.0	62.6	65.1	64.4	59.9
	* 4	67.7	65.1	63.3	62.9	60.5

* Infected Sheep without pair-fed control partners.

Appendix 4: Table 7

Experiment 1: Apparent Ash Digestibility Coefficients (%) in Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	31.8	34.2	46.5	56.5	
	6	27.1	36.3	55.9	57.3	
	962	30.1	35.5	50.1	53.0	
	Mean	29.7	35.3	50.8	55.6	
	S.E.	2.4	1.1	4.7	2.3	
	Infected					
	989	35.0	35.1	52.5	45.6	
	981	29.8	34.4	48.6	46.2	
	961	18.4	31.3	48.4	50.6	
	Mean	27.7	33.6	49.8	47.5	
	S.E.	8.5	2.0	2.3	2.7	
	+ 985	30.0	26.6	47.9	52.3	
	+ 992	22.0	37.4	59.6	48.9	
Hay + Compound Diet	Control					
	7	33.2	37.8	44.4	44.1	41.8
	952	42.9	44.6	48.9	50.9	
	964	53.9	51.1	54.8	55.5	54.9
	Mean	43.3	44.5	49.4	50.2	48.4
	S.E.	10.3	6.7	5.2	5.7	9.3
	Infected					
	5	44.3	44.8	48.6	52.4	37.7
	994	42.9	40.3	48.9	43.1	
	965	44.7	48.0	49.7	51.3	39.1
	Mean	44.0	44.4	49.1	48.9	38.4
	S.E.	0.9	3.9	0.6	5.1	1.0
	* 954	38.8	36.7	45.3	39.8	40.0
	* 4	46.4	43.3	53.7	48.7	43.2

* Infected sheep without pair-fed control partners.

Appendix 4: Table 8

Experiment 1: Apparent Crude Protein Digestibility Coefficients (%) in Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	39.6	43.0	51.0	41.9	
	6	37.4	47.3	52.1	43.8	
	962	36.8	41.8	45.7	37.0	
	Mean	37.9	44.0	49.6	40.9	
	S.E.	0.9	2.9	3.4	3.5	
	Infected					
	989	40.1	38.8	52.5	28.7	
	981	38.8	41.6	49.1	31.8	
	961	40.7	43.4	52.0	37.8	
	Mean	39.9	41.3	51.2	32.8	
	S.E.	0.6	2.3	1.8	4.6	
	* 985	46.5	40.3	55.8	34.0	
	* 992	35.4	36.1	50.9	31.1	
Hay + Compound Diet	Control					
	7	59.3	62.8	66.3	62.7	62.4
	952	59.5	56.6	62.2	59.6	
	964	61.6	63.0	67.1	60.4	64.7
	Mean	60.1	60.7	65.2	60.9	63.6
	S.E.	0.7	3.6	2.6	1.6	1.2
	Infected					
	5	60.6	61.7	65.7	64.6	62.9
	994	56.0	59.5	66.9	60.6	
	965	60.7	63.2	66.7	58.6	56.8
	Mean	59.1	61.5	66.4	61.3	59.9
	S.E.	1.6	1.9	0.6	3.1	3.1
	* 954	60.9	63.6	67.2	55.1	67.9
	* 4	68.4	64.7	63.4	56.9	68.4

* Infected sheep without pair-fed control partners

Appendix 4: Table 9

Experiment 1: Apparent Ether Extract Digestibility Coefficients (%) in Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection			
			5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	57.7	57.7	59.6	67.7	
	6	63.1	63.1	66.7	74.9	
	962	59.8	60.7	57.7	68.7	
	Mean	60.2	60.5	61.3	70.4	
	S.E.	1.6	1.6	2.7	2.3	
	Infected					
	989	61.0	60.4	66.2	80.0	
	981	67.7	68.0	57.0	64.2	
	961	63.0	62.7	55.6	69.2	
	Mean	63.9	63.7	59.6	71.1	
	S.E.	2.0	2.3	3.3	4.7	
	* 985	N.D.	N.D.	N.D.	N.D.	
	* 992	N.D.	N.D.	N.D.	N.D.	
Hay + Compound Diet	Control					
	7	78.5	79.3	76.0	89.3	84.0
	952	82.5	81.9	76.6	79.6	
	964	73.8	75.7	74.3	72.5	74.0
	Mean	78.3	79.0	75.6	80.5	79.0
	S.E.	2.5	1.8	0.7	4.9	5.0
	Infected					
	5	76.1	77.9	76.4	85.8	81.8
	994	75.2	78.7	74.3	78.6	
	965	74.9	78.3	78.5	64.9	69.9
	Mean	75.4	78.3	76.4	76.4	75.4
	S.E.	0.4	0.2	1.2	6.1	6.0
	* 954	N.D.	N.D.	N.D.	N.D.	N.D.
	* 4	N.D.	N.D.	N.D.	N.D.	N.D.

* Infected sheep without pair-fed control partners
N.D. Not determined

Appendix 4: Table 10

Experiment 1: Daily Water Intake (ml/g D.M. intake) of Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	1.79	2.11	2.85	2.22	
	6	1.62	1.47	2.10	1.47	
	962	1.70	1.71	2.07	1.81	
	Mean	1.70	1.76	2.34	1.83	
	S.E.	0.05	0.19	0.26	0.22	
	Infected					
	989	1.77	1.95	2.77	2.83	
	981	1.48	1.94	2.51	2.22	
	961	2.47	3.45	4.97	3.60	
	Mean	1.91	2.45	3.42	2.88	
	S.E.	0.29	0.50	0.78	0.40	
	* 985	1.73	2.22	4.37	2.61	
	* 992	1.75	1.96	3.43	2.87	
Hay + Compound Diet	Control					
	7	2.71	2.79	3.37	2.62	2.33
	952	3.00	3.08	4.33	3.42	
	964	2.13	2.35	2.90	2.39	2.60
	Mean	2.61	2.74	3.53	2.81	2.47
	S.E.	0.26	0.21	0.42	0.31	0.13
	Infected					
	5	2.50	2.99	3.71	3.18	3.27
	994	2.66	2.44	2.89	2.56	
	965	2.59	2.77	3.48	3.11	4.51
	Mean	2.58	2.73	3.36	2.95	3.90
	S.E	0.05	0.16	0.24	0.20	0.62
	* 954	2.28	2.85	3.35	3.02	3.30
	* 4	2.49	2.72	2.74	2.50	2.97

* Infected sheep without pair-fed control partners.

Appendix 4: Table 11

Experiment 1: Daily Faecal Water Output (ml/g D.M. intake) of Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	0.75	0.66	0.48	0.64	
	6	0.62	0.57	0.61	0.59	
	962	0.73	0.66	0.57	0.79	
	Mean	0.70	0.63	0.55	0.67	
	S.E.	0.04	0.03	0.04	0.06	
	Infected					
	989	0.74	0.72	0.57	0.89	
	981	0.46	0.63	0.66	0.74	
	961	0.76	0.85	0.63	0.86	
	Mean	0.65	0.73	0.62	0.83	
	S.E.	0.10	0.06	0.03	0.05	
	* 985	0.56	0.61	0.56	0.73	
	* 992	0.76	0.81	0.60	0.87	
Hay + Compound Diet	Control					
	7	0.91	0.97	0.76	0.93	0.95
	952	1.07	1.12	0.83	1.10	
	964	0.78	0.75	0.62	0.70	0.74
	Mean	0.92	0.95	0.74	0.91	0.85
	S.E.	0.08	0.11	0.06	0.12	0.10
	Infected					
	5	0.92	1.01	0.82	1.09	1.09
	994	0.85	1.04	0.76	0.91	
	965	0.84	0.93	0.72	0.90	1.19
	Mean	0.87	0.99	0.77	0.97	1.14
	S.E.	0.02	0.03	0.03	0.06	0.05
	* 954	1.08	1.09	0.79	1.13	1.38
	* 4	0.61	0.75	0.61	0.85	0.80

* Infected sheep without pair-fed control partners.

Appendix 4: Table 12

Experiment 1: Daily Urine Output (ml/g D.M. Intake) of Fluke-infected and Control sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection			
			5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	0.43	0.94	1.62	1.06	
	6	0.55	0.41	0.66	0.71	
	962	0.24	0.44	0.56	0.24	
	Mean	0.41	0.60	0.95	0.67	
	S.E.	0.09	0.17	0.34	0.24	
	Infected					
	989	0.41	0.60	1.53	1.19	
	981	0.11	0.50	0.79	0.58	
	961	0.86	1.60	3.00	1.86	
	Mean	0.46	0.90	1.77	1.21	
	S.E.	0.22	0.35	0.65	0.37	
	* 985	0.41	1.03	2.87	1.36	
	* 992	0.27	0.69	1.94	1.31	
Hay + Compound Diet	Control					
	7	1.01	1.06	1.83	1.04	0.79
	952	1.15	1.03	2.72	1.63	
	964	0.70	0.95	1.73	1.25	1.43
	Mean	0.95	1.01	2.09	1.31	1.11
	S.E.	0.13	0.05	0.31	0.17	0.32
	Infected					
	5	0.72	1.07	1.79	1.09	1.14
	994	0.95	0.57	1.01	0.87	
	965	0.90	1.02	1.84	1.33	2.57
	Mean	0.86	0.89	1.55	1.10	1.86
	S.E.	0.07	0.16	0.27	0.13	0.71
	* 954	0.50	0.80	1.32	0.87	1.09
	* 4	0.90	1.05	1.35	0.84	2.04

* Infected sheep without pair-fed control partners

Appendix 4: Table 13

Experiment 1: Apparent Daily Water Balance (ml/g D.M. intake) of Fluke-infected and Control Sheep

Diet	Sheep No.	Pre-Infection	Weeks Post-infection 5 - 7	8 - 10	12 - 14	18 - 20
Hay Only	Control					
	3	0.61	0.51	0.76	0.52	
	6	0.46	0.49	0.83	0.17	
	962	0.73	0.60	0.94	0.79	
	Mean	0.60	0.53	0.84	0.49	
	S.E.	0.08	0.03	0.05	0.18	
	Infected					
	989	0.62	0.63	0.67	0.75	
	981	0.91	0.81	1.05	0.91	
	961	0.85	1.00	1.34	0.88	
	Mean	0.79	0.81	1.02	0.85	
	S.E.	0.09	0.11	0.19	0.05	
	* 985	0.76	0.58	0.93	0.52	
	* 992	0.72	0.46	0.88	0.68	
Hay + Compound Diet	Control					
	7	0.79	0.76	0.78	0.66	0.60
	952	0.79	0.93	0.77	0.69	
	964	0.65	0.64	0.56	0.44	0.43
	Mean	0.74	0.78	0.70	0.60	0.52
	S.E.	0.05	0.08	0.07	0.08	0.08
	Infected					
	5	0.86	0.91	1.10	1.00	1.04
	994	0.86	0.82	1.13	0.78	
	965	0.85	0.82	0.92	0.89	0.76
	Mean	0.86	0.85	1.05	0.89	0.90
	S.E.	0.00	0.03	0.07	0.06	0.14
	* 954	0.70	0,96	1.24	1.03	0.82
	* 4	0.97	0.92	0.78	0.81	0.13

* Infected sheep without pair-fed control partners.

Appendix 4: Table 14

Experiment 2: Nitrogen Intake (g/day) of Fluke-infected and Control Sheep

Sheep No.	Pre-Infection	Weeks Post-infection								
		0 - 2	4 - 6	6 - 8	8 - 10	10 - 12	12 - 14	14 - 16	16 - 18	18 - 20
Control										
70	22.65	21.33	18.99	21.95	19.46	19.94	25.43	26.19	8.64	7.47
51	24.46	24.56	23.59	26.69	29.81	29.69	29.81	29.81	12.52	11.81
80	19.43	19.84	N.D.	15.19	19.67	21.59	21.64	19.84	8.16	7.27
98	17.19	18.32	18.95	16.50	19.24	22.96	26.51	26.26	6.85	5.89
83	23.18	24.19	23.81	25.66	28.96	29.81	29.81	29.81	11.14	9.05
97	24.19	24.63	24.68	26.36	29.70	29.81	29.81	29.81	11.59	10.19
Mean	21.85	22.15	22.00	22.06	24.47	25.63	27.17	26.95	9.82	8.61
S.E.	± 1.19	± 1.11	± 1.25	± 2.09	± 2.25	± 1.89	± 1.35	± 1.59	± 0.92	±0.88
Infected										
78	23.02	21.34	18.99	21.66	19.74	19.94	25.43	26.19	8.74	8.22
77	24.51	24.61	24.63	26.71	29.81	29.81	29.81	29.81	13.86	11.96
60	19.65	19.87	19.26*	14.81	19.67	21.59	21.64	19.84	8.08	8.01
68	17.23	18.32	18.98	16.23	19.24	22.96	26.51	26.26	6.24	4.80
54	23.60	24.34	23.85	25.38	28.96	29.81	29.81	29.81	11.84	9.09
61	24.67	24.70	24.70	26.69	29.70	29.81	29.81	29.81	12.43	12.45
Mean	22.11	22.20	22.23	21.91	24.52	25.65	27.17	26.95	10.20	9.09
S.E.	± 1.23	± 1.12	± 1.33	± 2.17	± 2.23	± 1.90	± 1.35	± 1.59	± 1.20	± 1.15

N.D. Not determined
* Not included in statistical analysis.

Appendix 4: Table 15

Experiment 2: Faecal Nitrogen Excretion (g/day) of Fluke-infected and Control Sheep

Sheep No.	Pre-Infection	Weeks Post-infection								
		0 - 2	4 - 6	6 - 8	8 - 10	10 - 12	12 - 14	14 - 16	16 - 18	18 - 20
Control										
70	6.04	6.05	4.68	5.29	5.73	6.30	6.10	6.27	3.24	4.33
51	7.84	7.67	7.02	7.38	0.08	7.68	8.03	7.53	7.17	6.69
80	5.96	6.18	N.D.	4.08	4.84	5.27	5.82	5.68	4.48	3.78
98	5.89	5.40	5.72	4.79	5.00	5.97	7.30	6.58	3.97	4.24
83	6.53	7.27	6.79	6.72	7.73	7.37	7.18	7.09	6.00	5.88
97	7.13	6.99	6.84	6.92	7.49	7.41	7.79	7.55	4.93	5.47
Mean	6.57	6.59	6.21	5.86	6.48	6.67	7.04	6.78	4.97	5.07
S.E.	± 0.32	± 0.35	± 0.45	± 0.54	± 0.59	± 0.39	± 0.37	± 0.30	± 0.58	± 0.46
Infected										
78	6.75	6.20	5.81	5.87	4.93	4.62	6.47	6.95	4.33	4.10
77	7.89	7.96	8.38	7.73	8.23	8.22	8.70	8.30	7.35	6.88
60	5.76	6.02	5.64*	3.88	4.92	5.49	5.57	5.33	4.14	5.36
68	6.04	5.17	5.44	4.83	5.61	6.39	6.61	7.14	5.75	4.09
54	7.20	7.21	7.11	6.91	7.32	7.94	7.80	8.36	6.08	4.96
61	7.62	7.68	6.86	7.01	8.00	7.31	7.15	7.80	6.48	6.42
Mean	6.88	6.71	6.72	6.04	6.50	6.66	7.05	7.31	5.69	5.30
S.E.	± 0.35	± 0.44	± 0.52	± 0.60	± 0.62	± 0.58	± 0.45	± 0.46	± 0.51	± 0.48

N.D. Not determined
* Not included in statistical analysis

Appendix 4: Table 16

Experiment 2: Urinary Nitrogen Excretion (g/day) of fluke-infected and control Sheep

Sheep No.	Pre-Infection	Weeks Post-infection								
		0 - 2	4 - 6	6 - 8	8 - 10	10 - 12	12 - 14	14 - 16	16 - 18	18 - 20
Control										
70	11.54	8.19	10.77	11.34	14.51	14.14	17.07	18.15	7.68	6.23
51	12.71	12.14	13.39	14.06	19.49	19.91	19.91	19.09	8.43	6.88
80	11.36	11.13	N.D.	8.85	13.79	14.92	13.79	14.72	7.22	5.36
98	9.11	9.16	9.59	6.03	11.81	13.63	13.14	14.19	5.68	4.33
83	10.80	11.53	12.46	12.88	17.59	18.24	18.11	18.64	7.79	5.91
97	12.61	13.17	12.68	13.16	16.81	17.73	17.54	17.85	6.54	4.78
Mean	11.36	10.89	11.78	11.05	15.67	16.49	16.59	17.11	7.22	5.58
S.E.	± 0.54	± 0.76	± 0.70	± 1.25	± 1.15	± 1.06	± 1.07	± 0.86	± 0.40	± 0.39
Infected										
78	11.34	13.45	11.98	12.31	18.84	15.02	18.43	18.89	8.49	5.79
77	12.23	12.93	13.24	12.39	20.87	20.41	20.15	21.16	10.41	8.79
60	10.86	11.51	11.86*	9.64	13.49	14.46	14.11	15.28	7.74	6.69
68	8.30	8.95	10.64	8.26	12.61	16.27	16.51	19.97	8.59	7.39
54	8.90	9.68	10.18	10.09	17.19	16.09	15.40	16.28	7.31	5.44
61	10.86	11.24	11.86	9.14	19.84	18.89	18.39	18.66	7.83	6.18
Mean	10.42	11.29	11.58	10.31	16.14	16.86	17.17	18.04	8.40	6.71
S.E.	± 0.61	± 0.72	± 0.54	± 0.69	± 1.50	± 0.95	± 0.91	± 0.85	± 0.45	± 0.50

N.D. not determined

* not included in statistical analysis

Appendix 4: Table 17

Experiment 2: Nitrogen Balance (g/day) of Fluke-infected and Control Sheep

Sheep No.	Pre-Infection	Weeks Post-infection								
		0 - 2	4 - 6	6 - 8	8 - 10	10 - 12	12 - 14	14 -16	16 - 18	18 - 20
Control										
70	+ 5.07	+ 7.09	+ 3.54	+ 5.32	- 0.78	- 0.50	+ 2.06	+ 1.77	- 2.28	- 3.09
51	+ 3.91	+ 4.75	+ 3.18	+ 5.25	+ 2.24	+ 2.10	+ 1.87	+ 3.19	- 3.08	- 1.76
80	+ 2.11	+ 2.53	N.D.	+ 2.26	+ 1.04	+ 1.40	+ 2.03	- 0.56	- 3.54	- 1.87
98	+ 2.19	+ 3.76	+ 3.64	+ 5.68	+ 2.43	+ 3.36	+ 6.07	+ 5.49	- 2.80	- 2.68
83	+ 5.85	+ 5.39	+ 4.56	+ 6.06	+ 3.64	+ 4.20	+ 4.52	+ 4.08	- 2.65	- 2.74
97	+ 4.45	+ 4.47	+ 5.16	+ 6.28	+ 5.40	+ 4.67	+ 4.48	+ 4.41	+ 0.12	- 0.06
Mean	+ 3.93	+ 4.67	+ 4.02	+ 5.14	+ 2.33	+ 2.54	+ 3.51	+ 3.06	- 2.37	- 2.03
S.E.	± 0.62	± 0.63	± 0.37	± 0.60	± 0.87	± 0.79	± 0.72	± 0.89	± 0.53	± 0.45
Infected										
78	+ 4.93	+ 1.69	+ 1.20	+ 3.48	+ 1.97	+ 0.30	+ 0.53	+ 0.35	- 4.08	- 1.67
77	+ 4.39	+ 3.72	+ 3.01	+ 6.59	+ 0.71	+ 1.18	+ 0.96	+ 0.35	- 3.90	- 3.71
60	+ 3.03	+ 2.34	+ 1.76*	+ 1.29	+ 1.26	+ 1.64	+ 1.96	- 0.77	- 3.80	- 4.04
68	+ 2.89	+ 4.20	+ 2.90	+ 3.14	+ 1.02	+ 0.30	+ 3.39	+ 1.15	- 8.10	- 6.68
54	+ 7.50	+ 7.45	+ 6.56	+ 8.38	+ 4.45	+ 5.78	+ 6.61	+ 5.17	- 1.55	- 1.31
61	+ 6.19	+ 5.78	+ 5.98	+ 10.54	+ 1.86	+ 3.61	+ 4.27	+ 3.35	- 1.88	- 0.15
Mean	+ 4.82	+ 4.20	+ 3.93	+ 5.57	+ 1.88	+ 2.14	+ 2.95	+ 1.60	- 3.89	- 2.93
S.E.	± 0.73	± 0.88	± 1.01	± 1.44	± 0.55	± 0.88	± 0.93	± 0.91	± 0.95	± 0.96

N.D. Not determined
* Not included in statistical analysis

Appendix 4: Table 18

Experiment 2: Apparent Dry Matter Digestibility Coefficients (%) in Fluke-infected and Control Sheep

Sheep No.	Pre-Infection	Weeks Post-Infection								
		0 - 2	4 - 6	6 - 8	8 - 10	10 - 12	12 - 14	14 - 16	16 - 18	18 - 20
Control										
70	62.2	60.7	64.6	61.4	55.6	60.7	62.0	59.9	44.9	46.9
51	56.4	56.3	59.0	55.3	60.4	57.2	57.5	57.4	39.9	39.7
80	58.4	60.1	N.D.	58.9	55.6	58.6	57.4	58.5	43.5	53.3
98	55.5	60.4	60.9	55.7	62.3	58.6	58.0	57.7	41.2	41.5
83	60.6	59.0	60.1	59.4	58.2	57.7	58.4	57.3	44.9	40.7
97	59.3	57.9	60.0	58.0	56.9	57.5	56.0	57.4	44.6	43.1
Mean	58.7	59.1	60.9	58.1	58.2	58.4	58.2	58.0	43.2	44.2
S.E.	±1.0	±0.7	±1.0	±0.9	±1.1	±0.5	±0.8	±0.4	±0.9	±2.0
Infected										
78	58.4	59.5	60.2	59.5	55.4	62.0	59.3	58.4	45.1	40.0
77	58.8	58.0	56.6	57.9	56.6	54.2	54.0	54.8	43.9	33.8
60	57.5	56.9	58.6*	61.4	61.1	60.7	60.1	57.4	40.5	38.6
68	52.6	60.7	59.7	56.4	60.0	56.5	58.8	58.4	25.5	28.1
54	57.6	57.9	58.7	59.0	60.0	58.4	58.4	56.3	45.6	34.0
61	58.7	58.0	61.7	60.3	59.1	59.2	59.6	58.0	43.0	31.1
Mean	57.3	58.5	59.4	59.1	58.7	58.5	58.4	57.2	40.6	34.3
SE.	±1.0	±0.6	±0.8	±0.7	±0.9	±1.2	±0.9	±0.6	±3.1	±1.8

N.D. Not determined
* Not included in statistical analysis

Appendix 4: Table 19

Experiment 2: Apparent Crude Protein Digestibility Coefficients (%) in Fluke-infected and Control Sheep

Sheep No.	Pre-Infection	Weeks Post-infection								
		0 - 2	4 - 6	6 - 8	8 - 10	10 - 12	12 - 14	14 - 16	16 - 18	18 - 20
Control										
70	73.3	71.6	75.4	75.9	70.6	68.4	76.0	76.1	62.5	42.1
51	68.0	68.8	70.2	72.4	72.9	74.1	73.1	74.7	42.7	43.3
80	69.3	68.8	N.D.	73.1	75.4	75.6	73.1	71.4	45.0	48.0
98	65.7	70.5	69.8	71.0	74.0	74.0	72.5	74.9	42.0	28.0
83	71.8	70.0	71.5	73.8	73.3	75.3	75.9	76.2	46.1	35.1
97	70.5	71.6	72.3	73.8	74.8	75.1	73.9	74.7	57.4	46.3
Mean	69.8	70.2	71.8	73.3	73.5	73.8	74.1	74.7	49.3	40.5
S.E.	±1.1	±0.5	±1.0	±0.7	±0.7	±1.1	±0.6	±0.7	±3.5	±3.1
Infected										
78	70.7	71.0	69.4	72.9	75.0	76.8	74.6	73.4	30.4	50.2
77	67.8	67.7	66.0	71.0	72.4	72.4	70.8	72.2	47.0	42.5
60	70.7	69.7	70.7*	73.8	75.0	74.6	74.3	73.1	48.8	33.1
68	65.0	71.8	71.3	70.2	70.9	72.2	75.1	72.8	7.9	14.8
54	69.5	70.4	70.2	72.8	74.7	73.4	73.8	72.0	48.7	45.4
61	69.1	68.9	72.2	73.7	73.1	75.5	76.0	73.8	47.9	48.5
Mean	68.8	69.9	69.8	72.4	73.5	74.2	74.1	72.9	41.8	39.1
S.E.	±0.9	±0.6	±1.1	±0.6	±0.7	±0.7	±0.7	±0.3	±6.8	±5.4

N.D. Not Determined
* Not included in statistical analysis

Appendix 4: Table 20

Experiment 2: Daily Water Intake (ml/g DM Intake) of Fluke-infected and Control Sheep

Sheep No.	Pre-Infection	Weeks Post-infection 0 - 2	4 - 6	6 - 8	8 - 10	10 - 12	12 - 14	14 - 16	16 - 18	18 - 20
Control										
70	2.46	2.65	2.96	2.89	3.79	3.72	3.26	3.82	3.57	3.00
51	1.46	3.03	3.42	3.59	4.03	4.44	4.61	5.52	4.73	4.02
80	2.50	2.38	N.D.	2.46	2.90	3.24	3.13	3.30	2.72	2.32
98	2.73	2.50	2.36	2.96	3.34	3.37	3.33	3.51	4.72	3.21
83	2.58	2.61	2.71	2.89	3.39	3.66	3.48	3.61	3.91	3.43
97	2.91	2.91	2.79	2.96	3.30	3.55	3.70	3.72	3.08	3.00
Mean	2.44	2.68	2.85	2.96	3.46	3.66	3.59	3.91	3.79	3.16
S.E.	±0.20	±0.10	±0.17	±0.14	±0.16	±0.17	±0.22	±0.33	±0.34	±0.23
Infected										
78	2.69	2.80	2.94	3.07	3.98	3.97	3.76	4.09	4.16	3.51
77	2.53	2.62	2.92	3.02	3.60	3.60	3.78	3.78	3.49	3.59
60	2.79	2.86	2.96*	3.03	3.48	3.42	3.54	4.00	5.68	5.15
68	3.12	3.00	3.32	4.26	4.40	4.45	4.20	5.07	13.31	5.73
54	2.69	2.62	2.85	2.86	3.11	3.46	3.38	3.72	3.38	3.77
61	2.97	3.02	3.16	2.37	3.73	4.16	3.97	4.71	4.85	4.20
Mean	2.80	2.82	3.04	3.10	3.72	3.84	3.77	4.23	5.81	4.33
S.E.	±0.08	±0.07	±0.09	±0.25	±0.18	±0.17	±0.12	±0.22	±1.54	±0.37

* Not included in statistical analysis
N.D. Not determined

Appendix 4: Table 21

Experiment 2: Apparent Daily Water Balance (ml/g D.M. Intake) of Fluke-infected and Control Sheep

Sheep No.	Pre-Infection	Weeks Post-infection 0 - 2	4 - 6	6 - 8	8 - 10	10 - 12	12 - 14	14 - 16	16 -18	18 - 20
Control										
70	0.51	0.58	0.53	0.47	0.53	0.65	0.71	0.76	0.81	0.29
51	0.62	0.79	0.85	0.71	0.92	0.81	1.20	1.03	1.18	0.59
80	0.58	0.48	N.D.	0.72	0.69	0.89	0.76	0.83	0.62	0.49
98	0.54	0.58	0.56	0.58	1.06	0.73	0.74	0.79	1.70	0.85
83	0.52	0.68	0.60	0.61	0.74	0.79	0.82	0.81	0.95	0.51
97	0.75	0.74	0.70	0.81	0.78	0.93	1.11	1.12	0.92	0.81
Mean	0.59	0.64	0.65	0.65	0.79	0.80	0.89	0.89	1.03	0.59
S.E.	± 0.03	± 0.04	± 0.05	± 0.04	± 0.07	±0.03	±0.08	± 0.05	± 0.15	± 0.08
Infected										
78	0.59	0.67	0.76	0.91	1.13	1.13	1.07	1.09	1.37	0.64
77	0.52	0.62	0.77	0.76	0.90	0.79	0.86	0.87	0.82	0.38
60	0.62	0.71	0.87*	0.77	0.97	0.92	1.11	1.21	1.35	0.59
68	0.58	0.79	0.83	0.88	0.95	0.96	0.95	1.12	2.29	0.44
54	0.56	0.51	0.73	0.72	0.69	0.80	0.92	0.93	0.86	0.76
61	0.61	0.62	0.78	0.79	0.82	1.01	1.10	1.33	1.23	0.62
Mean	0.58	0.65	0.77	0.81	0.91	0.94	1.00	1.10	1.32	0.57
S.E.	± 0.00	± 0.03	± 0.01	± 0.00	± 0.05	± 0.04	± 0.03	± 0.07	± 0.22	± 0.05

N.D. Not determined
* Not included in statistical analysis

APPENDIX 5

Data relevant to Chapter 5:

Observations on the Pathophysiological Changes Occurring in Sheep Exposed to Different Levels and Duration of Infection with S. mattheei

Appendix 5: Table 1

Packed Cell Volumes (%) of sheep exposed to two different levels of *S. mattheei* infection and worm-free controls.

Weeks After Infection	Group					
	Control		5,000		10,000	
	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.
3	13	34.9 ± 0.8	16	35.7 ± 0.7	11	34.3 ± 1.2
4	13	33.8 ± 1.1	16	35.0 ± 1.0	11	32.5 ± 1.2
5	13	32.3 ± 0.8	16	32.5 ± 0.8	11	30.3 ± 1.0
6	13	31.9 ± 0.8	16	31.0 ± 0.8	11	28.9 ± 0.7
7	13	30.6 ± 0.9	16	30.3 ± 0.7	11	28.7 ± 0.9
8	13	29.8 ± 0.8	16	28.6 ± 0.7	11	26.9 ± 1.0
9	13	29.2 ± 0.9	16	26.6 ± 0.6	11	25.0 ± 0.9
10	13	30.5 ± 0.7	16	27.2 ± 0.7	11	25.3 ± 0.7
11	13	30.4 ± 0.6	16	26.5 ± 0.7	11	23.8 ± 0.8
12	13	29.5 ± 0.7	12	26.5 ± 0.8	9	24.1 ± 1.2
13	13	29.7 ± 0.6	12	26.8 ± 0.9	9	24.2 ± 1.1
14	13	30.6 ± 0.6	12	27.7 ± 0.9	9	24.3 ± 1.2
15	13	29.5 ± 0.8	12	27.9 ± 1.2	9	24.1 ± 1.3
16	13	30.7 ± 0.4	12	27.7 ± 0.7	9	22.9 ± 1.3
17	13	30.0 ± 0.5	12	26.5 ± 0.8	9	22.9 ± 1.2
18	13	31.3 ± 0.6	12	27.3 ± 1.0	9	24.1 ± 1.0
19	13	30.6 ± 0.5	12	27.8 ± 0.9	9	23.9 ± 1.0
20	13	31.2 ± 0.6	12	28.5 ± 0.9	9	25.2 ± 0.9
21	13	32.0 ± 0.4	12	28.6 ± 1.0	9	26.7 ± 1.2
22	13	32.5 ± 0.6	12	28.1 ± 0.9	9	25.3 ± 1.5
23	13	32.0 ± 0.5	12	28.0 ± 0.7	9	25.1 ± 1.1
24	13	31.9 ± 0.7	12	28.0 ± 0.8	9	23.9 ± 1.5
25	13	31.8 ± 0.7	12	28.3 ± 0.9	9	24.6 ± 1.4
26	13	31.9 ± 0.6	9	28.3 ± 1.3	9	24.9 ± 1.3
27	13	32.2 ± 0.4	9	28.9 ± 1.3	9	25.1 ± 1.4
28	13	32.7 ± 0.5	9	29.3 ± 1.3	9	25.1 ± 1.7
29	13	32.2 ± 0.7	9	29.1 ± 1.2	9	25.3 ± 1.6

Appendix 5: Table 1 (continued)

Weeks After Infection	Group					
	Control		5,000		10,000	
	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.
30	13	32.9 ± 0.9	9	30.5 ± 1.4	9	26.6 ± 1.6
31	13	33.1 ± 0.8	9	28.9 ± 1.4	9	25.7 ± 1.6
32	13	33.2 ± 1.0	9	29.2 ± 1.7	9	24.8 ± 1.6
33	13	29.9 ± 0.7	9	25.9 ± 1.2	9	24.1 ± 2.1
34	13	31.0 ± 0.7	9	26.6 ± 1.4	9	22.7 ± 2.2
35	12	31.0 ± 0.7	9	26.4 ± 1.6	9	23.3 ± 2.1
36	12	31.6 ± 1.0	9	26.4 ± 1.6	9	22.8 ± 1.9
37	12	31.0 ± 0.6	9	26.5 ± 1.7	8	24.1 ± 1.4
38	12	32.4 ± 0.7	9	27.7 ± 1.7	8	24.9 ± 1.5
39	12	31.9 ± 0.6	9	27.8 ± 1.4	8	24.9 ± 1.3
40	12	31.4 ± 0.8	9	26.8 ± 1.7	8	24.4 ± 1.1
41	12	31.6 ± 0.8	8	27.8 ± 1.3	8	24.7 ± 1.4
42	12	32.0 ± 1.0	8	29.1 ± 1.4	8	24.9 ± 1.2
43	12	30.5 ± 0.6	8	28.2 ± 1.2	8	25.4 ± 1.0
44	12	32.0 ± 0.8	8	30.1 ± 1.2	8	26.6 ± 1.0
45	12	32.8 ± 0.9	8	30.8 ± 1.3	8	27.4 ± 1.1
46	12	31.8 ± 0.9	8	30.0 ± 1.4	8	28.0 ± 0.8
47	12	32.0 ± 0.9	8	29.7 ± 1.4	8	27.2 ± 1.1
48	12	32.5 ± 0.9	8	29.9 ± 1.2	8	28.1 ± 1.0
49	12	33.1 ± 1.1	8	30.6 ± 1.0	8	28.3 ± 1.0
50	12	32.8 ± 0.9	8	30.5 ± 0.8	8	28.6 ± 0.9
51	12	33.4 ± 0.9	8	30.9 ± 0.7	8	28.3 ± 1.1
52	12	32.6 ± 1.1	8	29.9 ± 0.6	8	28.4 ± 1.0
53	12	33.5 ± 1.0	8	30.8 ± 0.6	8	28.7 ± 1.2
54	12	32.8 ± 1.1	8	30.6 ± 0.6	8	27.9 ± 1.3
55	12	33.0 ± 1.1	8	30.9 ± 0.7	8	28.4 ± 1.0
56	12	32.9 ± 1.0	8	30.9 ± 0.9	8	28.2 ± 1.5
57	12	33.4 ± 1.0	8	31.2 ± 0.6	8	28.1 ± 1.3

Appendix 5: Table 2

Body Weights (kg) of sheep exposed to two different levels of S. mattheei infection and worm-free controls

Weeks After Infection	Group					
	Control		5,000		10,000	
	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.
3	11	35.4 ± 1.1	13	34.0 ± 1.1	11	38.6 ± 1.5
4	11	36.8 ± 1.4	13	35.3 ± 1.1	11	39.4 ± 1.7
5	11	38.5 ± 1.5	13	35.5 ± 1.2	11	40.6 ± 1.7
6	11	38.5 ± 1.6	13	36.5 ± 1.3	11	40.8 ± 1.6
7	11	39.4 ± 1.6	13	37.2 ± 1.3	11	41.9 ± 1.7
8	11	40.2 ± 1.9	13	37.3 ± 1.3	11	41.6 ± 1.7
9	11	40.4 ± 1.6	13	38.3 ± 1.5	11	43.0 ± 1.8
10	11	41.6 ± 1.9	13	38.2 ± 1.6	11	42.3 ± 1.7
11	11	41.5 ± 1.8	13	38.5 ± 1.5	11	42.5 ± 1.6
12	11	41.6 ± 1.7	9	37.7 ± 1.9	9	42.1 ± 2.1
13	11	41.6 ± 1.7	9	37.7 ± 1.8	9	42.1 ± 2.3
14	11	41.9 ± 1.8	9	37.7 ± 2.0	9	41.5 ± 2.3
15	11	42.9 ± 2.0	9	37.7 ± 1.9	9	42.1 ± 2.3
16	11	43.4 ± 1.7	9	38.6 ± 2.0	9	42.3 ± 2.4
17	11	43.9 ± 1.8	9	40.0 ± 2.1	9	43.1 ± 2.2
18	11	44.3 ± 1.7	9	39.9 ± 1.9	9	43.5 ± 2.2
19	11	45.5 ± 1.8	9	40.7 ± 1.9	9	44.2 ± 2.4
20	11	46.0 ± 1.7	9	41.4 ± 2.0	9	44.9 ± 2.5
21	11	46.5 ± 1.8	9	42.1 ± 2.2	9	45.3 ± 2.6
22	11	47.7 ± 1.9	9	42.3 ± 2.2	9	45.5 ± 2.8
23	11	48.3 ± 2.0	9	43.1 ± 2.2	9	46.2 ± 2.8
24	11	48.9 ± 2.0	9	43.9 ± 2.1	9	46.7 ± 2.9
25	11	49.6 ± 2.1	9	44.7 ± 2.3	9	47.4 ± 3.0
26	11	50.2 ± 2.1	9	45.1 ± 2.4	9	48.1 ± 3.1
27	11	50.8 ± 2.3	9	44.9 ± 2.1	9	48.0 ± 3.1
28	11	50.5 ± 2.0	9	44.7 ± 2.0	9	46.8 ± 2.8
29	11	49.4 ± 1.9	9	43.0 ± 1.6	9	45.2 ± 2.6
30	11	50.1 ± 1.9	9	43.7 ± 1.7	9	45.0 ± 2.7
32	11	51.4 ± 1.9	8	46.6 ± 2.1	9	46.3 ± 3.0
34	11	52.6 ± 2.0	8	47.5 ± 2.0	9	47.4 ± 3.2
36	11	54.5 ± 1.9	8	48.6 ± 2.0	9	48.3 ± 3.4
38	11	54.6 ± 2.0	8	49.5 ± 2.1	8	51.7 ± 2.7
40	11	55.9 ± 2.0	8	50.4 ± 2.1	8	52.7 ± 2.7
42	11	56.9 ± 2.1	8	51.3 ± 2.2	8	53.8 ± 2.8
44	11	57.8 ± 2.2	8	52.6 ± 2.2	8	54.6 ± 2.8
46	11	58.2 ± 2.3	8	53.5 ± 2.3	8	55.4 ± 3.0
48	11	60.2 ± 2.4	8	54.4 ± 2.4	8	56.0 ± 3.2
50	11	61.4 ± 2.5	8	54.7 ± 2.7	8	57.0 ± 3.5
52	11	62.4 ± 2.6	8	56.1 ± 2.7	8	57.2 ± 3.7
54	11	63.3 ± 2.5	8	57.1 ± 2.6	8	58.2 ± 4.1
56	11	63.5 ± 2.6	8	57.8 ± 2.7	8	58.6 ± 4.2

Appendix 5: Table 3

Total Serum Protein Concentrations (g/100ml) of sheep exposed to two different levels of *S. mattheei* infection and worm-free controls

Weeks After Infection	Group					
	Control		5,000		10,000	
	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.
3	10	5.08 ± 0.21	13	5.14 ± 0.09	8	5.32 ± 0.09
4	10	4.97 ± 0.19	13	5.05 ± 0.10	8	5.39 ± 0.12
5	10	4.82 ± 0.11	13	5.16 ± 0.11	8	5.34 ± 0.12
6	10	4.97 ± 0.14	13	5.30 ± 0.14	8	5.23 ± 0.12
7	10	4.92 ± 0.12	13	5.18 ± 0.12	8	5.24 ± 0.14
8	10	5.11 ± 0.16	13	5.55 ± 0.11	8	5.34 ± 0.09
9	10	5.11 ± 0.15	13	5.32 ± 0.13	8	5.23 ± 0.10
10	10	5.52 ± 0.18	13	5.39 ± 0.13	8	5.33 ± 0.16
11	10	5.45 ± 0.15	13	5.36 ± 0.14	8	5.43 ± 0.17
12	10	5.11 ± 0.11	9	5.43 ± 0.16	6	5.44 ± 0.13
13	10	5.45 ± 0.10	9	5.71 ± 0.14	6	5.66 ± 0.14
14	10	5.58 ± 0.10	9	5.85 ± 0.17	6	6.08 ± 0.18
15	10	5.56 ± 0.08	9	5.85 ± 0.15	6	5.88 ± 0.15
16	10	5.22 ± 0.09	9	5.61 ± 0.12	6	5.94 ± 0.24
17	10	5.12 ± 0.10	9	5.69 ± 0.09	6	5.79 ± 0.24
18	10	5.47 ± 0.12	9	5.70 ± 0.18	6	6.04 ± 0.17
19	10	5.68 ± 0.12	9	6.00 ± 0.18	6	6.41 ± 0.11
20	10	5.57 ± 0.19	9	6.17 ± 0.15	6	6.35 ± 0.18
21	10	5.91 ± 0.18	9	6.14 ± 0.23	6	6.64 ± 0.23
22	10	5.80 ± 0.18	9	6.22 ± 0.20	6	6.60 ± 0.27
23	10	5.84 ± 0.20	9	6.22 ± 0.19	6	6.84 ± 0.24
24	10	5.70 ± 0.21	9	6.15 ± 0.21	6	6.59 ± 0.26
25	10	5.81 ± 0.15	9	6.11 ± 0.19	6	6.65 ± 0.22
26	10	5.82 ± 0.14	9	6.18 ± 0.21	6	6.75 ± 0.28
27	10	5.86 ± 0.05	7	6.43 ± 0.20	6	6.68 ± 0.24
28	10	5.78 ± 0.08	7	6.15 ± 0.25	6	6.59 ± 0.19
29	10	5.84 ± 0.11	7	6.32 ± 0.18	6	6.71 ± 0.24
30	10	5.68 ± 0.14	7	6.01 ± 0.17	6	6.68 ± 0.26
32	10	5.77 ± 0.13	6	6.24 ± 0.20	6	6.61 ± 0.24
34	10	5.82 ± 0.09	6	6.28 ± 0.13	6	6.48 ± 0.26
36	10	5.64 ± 0.12	6	6.39 ± 0.23	6	6.50 ± 0.21
38	10	5.78 ± 0.13	6	6.22 ± 0.10	6	6.66 ± 0.20
40	10	5.67 ± 0.15	6	6.43 ± 0.17	6	6.39 ± 0.20
42	10	5.73 ± 0.13	6	6.17 ± 0.15	6	6.33 ± 0.19
44	10	5.71 ± 0.15	6	6.19 ± 0.20	6	6.33 ± 0.28
46	10	5.72 ± 0.12	6	6.17 ± 0.18	6	6.28 ± 0.22
48	10	5.74 ± 0.18	6	6.07 ± 0.16	6	6.26 ± 0.20
50	10	5.86 ± 0.10	6	6.13 ± 0.21	6	6.29 ± 0.31
52	10	5.80 ± 0.10	6	6.14 ± 0.22	6	6.32 ± 0.23
54	10	5.79 ± 0.12	6	6.06 ± 0.23	6	6.17 ± 0.20
56	10	5.68 ± 0.16	6	6.15 ± 0.17	6	6.10 ± 0.21

Appendix 5: Table 4

Serum Albumin Concentrations (g/100ml) of sheep exposed to two different levels of *S. mattheei* infection and worm-free controls

Weeks After Infection	Group					
	Control		5,000		10,000	
	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.
3	10	2.68 ± 0.11	13	2.77 ± 0.11	8	2.68 ± 0.09
4	10	2.73 ± 0.12	13	2.66 ± 0.09	8	2.70 ± 0.14
5	10	2.53 ± 0.11	13	2.62 ± 0.09	8	2.55 ± 0.11
6	10	2.77 ± 0.11	13	2.77 ± 0.11	8	2.67 ± 0.08
7	10	2.90 ± 0.09	13	2.76 ± 0.10	8	2.62 ± 0.09
8	10	3.02 ± 0.08	13	2.77 ± 0.11	8	2.65 ± 0.08
9	10	2.92 ± 0.12	13	2.65 ± 0.10	8	2.49 ± 0.07
10	10	3.22 ± 0.15	13	2.85 ± 0.09	8	2.79 ± 0.09
11	10	3.36 ± 0.10	13	2.98 ± 0.11	8	2.93 ± 0.09
12	10	3.06 ± 0.10	9	2.99 ± 0.11	6	2.69 ± 0.04
13	10	3.30 ± 0.12	9	3.18 ± 0.08	6	2.68 ± 0.11
14	10	3.33 ± 0.07	9	3.22 ± 0.10	6	2.86 ± 0.19
15	10	3.32 ± 0.10	9	3.18 ± 0.05	6	2.77 ± 0.28
16	10	3.24 ± 0.10	9	3.06 ± 0.08	6	2.67 ± 0.23
17	10	2.97 ± 0.09	9	2.93 ± 0.04	6	2.71 ± 0.18
18	10	3.17 ± 0.08	9	3.05 ± 0.07	6	2.87 ± 0.13
19	10	3.46 ± 0.12	9	3.37 ± 0.07	6	3.10 ± 0.19
20	10	3.41 ± 0.07	9	3.36 ± 0.07	6	3.11 ± 0.18
21	10	3.51 ± 0.10	9	3.26 ± 0.08	6	3.05 ± 0.16
22	10	3.32 ± 0.07	9	3.23 ± 0.11	6	3.06 ± 0.11
23	10	3.43 ± 0.09	9	3.16 ± 0.09	6	3.07 ± 0.16
24	10	3.38 ± 0.05	9	3.26 ± 0.08	6	3.11 ± 0.16
25	10	3.36 ± 0.06	9	3.21 ± 0.10	6	3.12 ± 0.10
26	10	3.33 ± 0.06	9	3.16 ± 0.10	6	3.07 ± 0.12
27	10	3.24 ± 0.10	7	3.06 ± 0.09	6	2.89 ± 0.17
28	10	3.35 ± 0.13	7	2.91 ± 0.07	6	3.09 ± 0.13
29	10	3.26 ± 0.11	7	3.19 ± 0.11	6	3.04 ± 0.09
30	10	3.24 ± 0.13	7	2.92 ± 0.06	6	3.02 ± 0.09
32	10	3.26 ± 0.12	6	3.03 ± 0.12	6	2.98 ± 0.10
34	10	3.22 ± 0.11	6	3.10 ± 0.11	6	2.94 ± 0.15
36	10	3.22 ± 0.08	6	3.08 ± 0.09	6	3.03 ± 0.18
38	10	3.42 ± 0.07	6	3.08 ± 0.13	6	3.12 ± 0.09
40	10	3.41 ± 0.09	6	3.21 ± 0.08	6	2.89 ± 0.08
42	10	3.52 ± 0.09	6	3.12 ± 0.08	6	2.92 ± 0.16
44	10	3.35 ± 0.07	6	3.09 ± 0.09	6	2.96 ± 0.09
46	10	3.29 ± 0.14	6	3.06 ± 0.13	6	2.84 ± 0.10
48	10	3.40 ± 0.12	6	3.05 ± 0.18	6	2.95 ± 0.12
50	10	3.25 ± 0.10	6	2.92 ± 0.15	6	2.92 ± 0.10
52	10	3.37 ± 0.09	6	3.06 ± 0.10	6	2.94 ± 0.03
54	10	3.33 ± 0.10	6	3.01 ± 0.12	6	3.01 ± 0.06
56	10	3.25 ± 0.09	6	2.97 ± 0.10	6	2.86 ± 0.09

Appendix 5: Table 5

Serum Globulin Concentrations (g/100ml) of sheep exposed to two different levels of *S. mattheei* infection and worm-free controls

Weeks After Infection	Group					
	Control		5,000		10,000	
	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.	No. of Sheep	Mean ± S.E.
3	10	2.40 ± 0.18	13	2.37 ± 0.10	8	2.65 ± 0.07
4	10	2.24 ± 0.20	13	2.39 ± 0.10	8	2.69 ± 0.13
5	10	2.29 ± 0.11	13	2.54 ± 0.14	8	2.79 ± 0.15
6	10	2.19 ± 0.17	13	2.53 ± 0.16	8	2.56 ± 0.14
7	10	2.02 ± 0.17	13	2.42 ± 0.18	8	2.62 ± 0.13
8	10	2.09 ± 0.20	13	2.78 ± 0.17	8	2.73 ± 0.12
9	10	2.20 ± 0.16	13	2.68 ± 0.16	8	2.74 ± 0.11
10	10	2.30 ± 0.18	13	2.54 ± 0.14	8	2.54 ± 0.13
11	10	2.09 ± 0.18	13	2.39 ± 0.15	8	2.50 ± 0.14
12	10	2.05 ± 0.18	9	2.44 ± 0.20	6	2.75 ± 0.12
13	10	2.16 ± 0.17	9	2.53 ± 0.14	6	2.99 ± 0.13
14	10	2.25 ± 0.11	9	2.62 ± 0.21	6	3.23 ± 0.28
15	10	2.23 ± 0.11	9	2.67 ± 0.16	6	3.11 ± 0.34
16	10	1.98 ± 0.15	9	2.55 ± 0.14	6	3.28 ± 0.40
17	10	2.14 ± 0.07	9	2.86 ± 0.09	6	3.25 ± 0.56
18	10	2.30 ± 0.12	9	2.64 ± 0.21	6	3.17 ± 0.30
19	10	2.23 ± 0.12	9	2.63 ± 0.18	6	3.32 ± 0.29
20	10	2.16 ± 0.19	9	2.81 ± 0.10	6	3.24 ± 0.34
21	10	2.40 ± 0.13	9	2.87 ± 0.21	6	3.58 ± 0.31
22	10	2.48 ± 0.18	9	2.99 ± 0.19	6	3.54 ± 0.36
23	10	2.41 ± 0.21	9	3.06 ± 0.18	6	3.77 ± 0.21
24	10	2.32 ± 0.20	9	2.89 ± 0.18	6	3.48 ± 0.29
25	10	2.46 ± 0.16	9	2.90 ± 0.15	6	3.52 ± 0.28
26	10	2.49 ± 0.14	9	3.02 ± 0.17	6	3.68 ± 0.36
27	10	2.62 ± 0.13	7	3.37 ± 0.17	6	3.79 ± 0.36
28	10	2.44 ± 0.13	7	3.25 ± 0.22	6	3.50 ± 0.27
29	10	2.58 ± 0.16	7	3.13 ± 0.19	6	3.67 ± 0.32
30	10	2.45 ± 0.17	7	3.09 ± 0.17	6	3.66 ± 0.33
32	10	2.50 ± 0.19	6	3.22 ± 0.11	6	3.64 ± 0.28
34	10	2.60 ± 0.17	6	3.18 ± 0.12	6	3.54 ± 0.35
36	10	2.42 ± 0.11	6	3.31 ± 0.20	6	3.47 ± 0.30
38	10	2.36 ± 0.16	6	3.14 ± 0.17	6	3.54 ± 0.27
40	10	2.26 ± 0.18	6	3.22 ± 0.22	6	3.50 ± 0.26
42	10	2.21 ± 0.19	6	3.04 ± 0.22	6	3.41 ± 0.30
44	10	2.36 ± 0.19	6	3.10 ± 0.25	6	3.37 ± 0.28
46	10	2.43 ± 0.22	6	3.12 ± 0.26	6	3.44 ± 0.30
48	10	2.32 ± 0.26	6	3.03 ± 0.26	6	3.32 ± 0.28
50	10	2.58 ± 0.17	6	3.21 ± 0.34	6	3.38 ± 0.35
52	10	2.43 ± 0.12	6	3.01 ± 0.28	6	3.38 ± 0.25
54	10	2.46 ± 0.17	6	3.05 ± 0.33	6	3.16 ± 0.23
56	10	2.44 ± 0.18	6	3.18 ± 0.26	6	3.24 ± 0.29

Appendix 5: Table 6

Mean Blood Volumes (± S.E.) Of sheep exposed to two different levels of *S. mattheei* infection and worm-free controls

Weeks After Infection	Group	No. of Sheep	Plasma Volume (ml)	Plasma Volume (ml/kg)	Red Cell Volume (ml)	Red Cell Volume (ml/kg)	Blood Volume (ml)	Blood Volume (ml/kg)
3	10,000c.	8	1955 ± 74	45.5 ± 1.4	805 ± 39	18.8 ± 0.9	2760 ± 87	64.2 ± 1.7
	5,000c.	13	1698 ± 42	45.1 ± 1.2	794 ± 32	21.1 ± 0.9	2492 ± 63	66.1 ± 1.8
	Control	11	1676 ± 81	45.0 ± 1.9	761 ± 31	20.4 ± 0.7	2437 ± 96	65.4 ± 2.1
7	10,000c.	8	2264 ± 87	50.5 ± 1.2	756 ± 30	17.1 ± 1.2	3020 ± 82	67.6 ± 2.1
	5,000c.	13	1925 ± 53	47.8 ± 1.4	733 ± 35	18.2 ± 0.9	2659 ± 69	66.0 ± 2.2
	Control	11	1881 ± 82	44.6 ± 1.6	677 ± 28	16.2 ± 0.9	2558 ± 93	60.8 ± 2.2
12	10,000c.	8	2398 ± 118	55.5 ± 1.8	685 ± 46	16.1 ± 1.3	3082 ± 107	71.6 ± 2.0
	5,000c.	13	2111 ± 70	52.2 ± 1.8	690 ± 34	17.1 ± 0.9	2790 ± 91	69.0 ± 2.4
	Control	10	1963 ± 52	44.7 ± 1.4	656 ± 32	14.9 ± 0.7	2618 ± 77	59.7 ± 1.9
30	10,000c.	4	2732 ± 241	54.5 ± 3.7	876 ± 50	17.6 ± 0.8	3607 ± 278	72.0 ± 4.1
	5,000c.	7	2374 ± 124	49.8 ± 2.3	791 ± 28	16.6 ± 0.5	3164 ± 121	66.4 ± 2.1
	Control	10	2250 ± 132	43.2 ± 1.8	890 ± 65	17.0 ± 0.7	3140 ± 188	60.3 ± 2.2
57	10,000c.	6	2661 ± 112	43.4 ± 1.8	923 ± 61	15.0 ± 0.8	3584 ± 160	58.4 ± 2.4
	5,000c.	6	2542 ± 86	43.1 ± 1.1	801 ± 42	13.6 ± 0.5	3343 ± 103	56.6 ± 0.9
	Control	3	2396 ± 18	39.8 ± 0.1	943 ± 54	15.7 ± 0.9	3339 ± 41	55.5 ± 0.8

APPENDIX 6

Data relevant to Chapter 6:

Comparison of the Sequential Development of Disease in Sheep Infected with Different Strains of S. mattheei

Appendix 6: Table 1

Faecal Egg Counts (eggs/g) of sheep infected with two different strains of *S. mattheei*

Group	Sheep No.	Days After Infection								
		35	42	50	57	61	75	82	86	91
VW Strain	63	0	0	100	50	60	Dead			
	64	0	0	0	20	110	170	60	210	40
	65	0	0	13	30	80	220	110	590	250
	66	0	0	0	90	130	70	40	460	60
	67	0	0	0	216	150	80	40	210	420
	Mean	0	0	23	81	106	135	63	368	193
	S.E.	0	0	20	36	16	36	17	95	89
MT Strain	68	0	0	0	0	0	20	0	20	33
	69	0	0	0	0	0	0	0	50	10
	70	0	0	0	0	0	Dead			
	71	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0
	Mean	0	0	0	0	0	5	0	18	11
	S.E.	0	0	0	0	0	5	0	12	8

Appendix 6: Table 2

Body Weight (kg) changes in sheep following infection with two different strains of S. mattheei

Group	Sheep No.	Days After Infection																
		0	7	14	21	28	35	42	50	56	61	65	75	79	82	86	89	91
VW Strain	63	35	37	37	34	39	37	38	37	35	36	Dead						
	64	30	30	31	29	33	34	31	30	32	32	31	33	32	30	30	30	31
	65	36	36	36	35	41	37	39	38	37	37	37	39	39	37	37	35	35
	66	34	33	33	31	35	34	34	34	31	35	30	34	33	33	33	33	32
	67	36	37	37	31	35	36	37	36	36	37	34	39	36	35	34	34	35
	Mean	34	35	35	32	37	36	36	35	34	35	33	36	35	34	34	33	33
	S.E.	1	1	1	1	1	2	1	1	1	1	2	2	2	1	1	1	1
MT Strain	68	33	33	33	30	33	34	33	33	33	34	30	35	34	34	32	34	33
	69	39	39	38	36	39	39	39	38	39	41	39	43	42	40	41	40	41
	70	31	32	32	29	33	32	31	31	31	32	Dead						
	71	32	32	34	31	34	33	35	35	36	37	35	39	38	37	38	38	39
	72	38	38	40	35	37	37	39	40	37	40	38	41	41	41	41	41	44
	Mean	35	35	35	32	35	35	35	35	35	37	36	40	39	38	38	38	39
	S.E.	2	2	2	1	1	1	2	2	1	2	2	2	2	2	2	2	2
Control	73	36	36	36	33	39	37	38	38	38	40	40	43	43	41	42	42	42
	74	27	29	29	27	30	30	31	30	31	33	30	33	33	32	32	32	33
	75	34	35	34	32	37	35	35	35	36	37	37	36	37	37	37	38	38
	Mean	32	33	33	31	35	34	35	34	35	37	36	37	38	37	37	37	38
	S.E.	3	2	2	2	3	2	2	2	2	2	3	3	3	3	3	3	3

Appendix 6: Table 3

Packed Cell Volume (%) changes in sheep following infection with two different strains of *S. mattheei*

Group	Sheep No.	Days After Infection																
		0	7	14	21	28	35	42	50	56	61	65	75	79	82	86	89	91
VW Strain	63	32.3	31.3	30.3	30.3	28.3	28.3	30.3	28.3	15.2	13.1	Dead						
	64	32.3	32.3	33.3	35.4	31.3	30.3	32.3	29.3	28.3	25.2	25.3	20.2	22.2	22.2	20.2	16.2	20.2
	65	32.3	33.3	31.3	31.3	29.3	27.3	30.3	30.3	25.3	22.2	21.2	18.2	21.2	19.2	17.2	17.2	16.2
	66	37.4	34.3	35.4	34.3	33.3	33.3	33.3	34.3	31.3	25.3	27.3	22.2	22.2	22.2	20.2	20.2	20.2
	67	35.4	35.4	36.4	35.4	35.3	32.3	34.3	32.3	34.3	28.3	30.3	25.3	26.3	25.3	22.2	24.2	24.2
	Mean	33.9	33.3	33.3	33.3	31.5	30.3	32.1	30.9	26.9	22.8	26.0	21.5	23.0	22.2	20.0	19.5	20.2
	S.E.	1.1	0.7	1.2	1.1	1.3	1.1	0.8	1.1	3.3	2.6	1.9	1.5	1.1	1.2	1.0	1.8	1.6
MT Strain	68	37.4	36.4	36.4	34.3	36.4	31.3	33.3	32.3	33.3	30.3	31.3	29.3	28.3	32.3	25.3	33.3	27.3
	69	33.3	35.4	35.4	33.3	34.3	31.3	34.3	31.3	31.3	31.2	30.3	26.3	26.3	29.3	26.3	27.3	25.3
	70	29.3	31.3	32.3	30.3	30.3	26.3	30.3	27.8	27.3	27.3	Dead						
	71	31.3	32.3	32.3	32.3	32.3	31.3	32.3	32.3	28.3	27.3	30.3	28.3	27.3	28.3	24.3	25.3	27.3
	72	33.3	34.3	36.4	34.3	37.4	32.3	34.4	31.3	32.3	28.3	32.3	32.3	27.3	28.3	28.3	28.3	28.3
	Mean	32.9	33.9	34.6	32.9	34.1	30.5	32.9	31.0	30.5	28.9	31.1	29.1	27.3	29.6	26.1	28.6	27.1
	S.E.	1.3	0.9	0.9	0.7	1.3	1.1	0.8	0.8	1.2	0.8	0.5	1.3	0.4	0.9	0.9	1.7	0.6
Control	73	32.3	34.3	33.3	31.3	30.3	32.3	34.3	30.3	30.3	30.3	29.3	28.3	29.3	32.3	28.3	28.3	29.3
	74	34.3	34.3	36.4	34.3	35.4	34.3	34.4	33.3	31.3	29.3	30.3	29.3	33.3	35.3	29.3	30.3	29.3
	75	30.3	33.3	33.3	32.3	32.3	31.3	34.3	33.3	33.3	32.3	32.3	31.3	30.3	33.2	32.3	30.3	30.3
	Mean	32.3	34.0	34.3	32.6	32.7	32.6	34.3	32.3	31.6	30.6	30.6	29.6	31.0	33.6	30.0	29.6	29.6
	S.E.	1.2	0.3	1.0	0.9	1.5	0.9	0.0	1.0	0.9	0.9	0.9	0.9	1.2	0.9	1.2	0.7	0.3

Appendix 6: Table 4

Plasma Volumes (ml/kg) of sheep infected with two different strains of *S. mattheei* and worm-free controls

Group	Sheep No.	Days After Infection			
		10	41	60	83
VW Strain	63	44.8	53.1	29.4+	Dead
	64	42.2	46.1	55.9	72.2
	65	46.6	46.8	58.9	68.3
	66	41.9	40.1	53.3	59.9
	67	42.6	45.5	52.7	79.8
	Mean	43.6	46.3	55.2	70.1
	S.E.	0.9	2.0	1.4	4.1
MT Strain	68	44.5	46.3	42.6	50.5
	69	42.6	44.1	44.3	46.5
	70	43.3	45.9	46.1	Dead
	71	44.5	44.0	42.2	44.8
	72	42.0	43.6	43.9	47.1
	Mean	43.4	44.8	43.8	47.2
	S.E.	0.5	0.5	0.7	1.2
Control	73	44.1	41.0	42.8	42.6
	74	45.2	44.7	41.5	43.1
	75	46.1	43.7	39.8	43.0
	Mean	45.1	43.1	41.4	42.9
	S.E.	0.6	1.1	0.9	0.2

+ Not included in the mean

Appendix 6: Table 5

Red Cell Volumes (ml/kg) of sheep infected with two different strains of S. mattheei and worm-free controls

Group	Sheep No.	Days After Infection			
		10	41	60	83
VW Strain	63	15.5	18.2	5.0+	Dead
	64	15.3	17.9	18.1	15.5
	65	14.7	16.7	17.6	13.9
	66	15.7	19.7	20.2	15.0
	67	17.9	21.4	21.5	22.3
	Mean	15.8	18.8	19.4	16.7
	S.E.	0.5	0.8	0.9	1.9
MT Strain	68	17.5	20.8	18.7	18.0
	69	14.8	20.0	19.4	16.2
	70	14.2	17.4	15.8	Dead
	71	18.8	18.0	17.6	14.9
	72	18.1	19.6	19.7	17.3
	Mean	16.7	19.2	18.2	16.6
	S.E.	0.9	0.6	0.7	0.7
Control	73	15.8	17.6	17.9	14.6
	74	18.9	22.5	18.7	17.2
	75	17.7	19.4	18.7	17.2
	Mean	17.5	19.8	18.4	16.3
	S.E.	0.9	1.4	0.3	0.9

+ Not included in the mean

Appendix 6: Table 6

Plasma Iron Turnover Rate (mg/day) in sheep infected with two different strains of *S. mattheei* and worm-free controls

Group	Sheep No.	Days After Infection			
		10	41	60	83
VW Strain	63	8.52	12.07	10.86	Dead
	64	7.92	5.30	4.94	6.16
	65	7.50	6.26	10.16	8.80
	66	11.59	7.54	8.18	10.90
	67	15.92	10.78	5.64	8.37
	Mean	10.29	8.39	7.96	8.56
	S.E.	1.58	1.31	1.18	0.97
MT Strain	68	16.65	9.31	8.65	9.75
	69	8.95	8.40	10.29	14.30
	70	9.13	6.38	6.57	Dead
	71	12.88	10.41	10.32	11.69
	72	11.73	13.06	8.64	7.36
	Mean	11.87	9.51	8.89	10.78
	S.E.	1.41	1.11	0.69	1.47
Control	73	7.75	7.43	13.74	10.54
	74	13.76	7.78	11.32	7.91
	75	8.70	8.72	12.28	10.18
	Mean	10.07	7.98	12.45	9.54
	S.E.	1.87	0.39	0.70	0.82

APPENDIX 7

Data relevant to Chapter 7:

Immunization of Sheep Against a Virulent Strain of S. mattheei, Using a Strain of S. mattheei Attenuated by Hamster Passage

Appendix 7. Table 1

Body Weights (kg), following a challenge infection with a virulent strain of S. mattheei, in sheep previously exposed to a non-virulent strain of S. mattheei and worm-free sheep

Group	Sheep No.	Weeks After Challenge															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control	55	-	71.7	72.1	73.9	74.8	74.4	72.6	76.2	75.7	80.3	80.7	79.4	77.1	72.1	78.5	78.9
	51	-	61.7	62.6	63.5	63.0	61.2	60.8	63.5	63.9	66.7	66.7	68.9	63.0	63.0	65.3	65.3
	32	-	74.8	73.9	75.7	76.6	75.3	78.0	79.8	81.6	84.4	83.9	84.8	81.2	79.4	85.3	79.8
	8	-	75.7	77.1	78.0	79.8	80.3	80.3	84.8	81.6	86.2	86.6	87.1	83.9	79.8	85.3	79.4
	Mean	-	71.0	71.4	72.8	73.6	72.8	72.9	76.1	75.7	79.4	79.5	80.1	76.3	73.6	78.6	75.9
	S.E.	-	3.2	3.1	3.2	3.7	4.1	4.4	4.5	4.2	4.4	4.4	4.1	4.6	3.9	4.7	3.5
Vaccinates	7	-	76.6	77.1	78.5	79.8	78.9	77.5	80.3	80.7	83.4	80.3	81.6	81.6	75.3	81.2	76.2
	52	-	66.7	67.1	68.0	66.7	67.6	68.0	71.7	68.9	74.4	71.2	72.6	72.6	68.9	72.6	72.1
	60	-	57.1	58.5	59.9	61.7	62.6	59.9	59.9	63.5	62.6	62.1	63.0	62.1	58.0	60.8	60.3
	19	-	58.0	58.5	59.4	61.7	61.2	60.8	63.5	61.7	61.7	59.9	60.3	59.4	57.6	59.9	62.6
	Mean	-	64.6	65.3	66.5	67.5	67.6	66.6	68.9	68.7	70.5	68.4	69.4	68.9	65.0	68.6	67.8
	S.E.	-	4.5	4.4	4.5	4.3	4.0	4.1	4.5	4.3	5.2	4.7	4.9	5.1	4.3	5.1	3.8
Non-Vaccinates	11	-	58.5	59.9	60.8	62.6	61.2	59.0	57.1	57.1	54.4	54.0	52.6	49.4	49.0	48.1	49.4
	95	-	66.2	67.1	68.5	70.7	68.9	67.1	63.0	63.5	64.4	62.6	63.0	59.4	55.3	57.6	58.0
	68	-	61.7	61.2	64.4	65.3	62.6	62.6	63.5	60.3	62.6	60.3	59.4	55.8	54.9	53.5	57.1
	190	-	59.9	59.0	61.2	63.0	61.7	60.8	61.7	59.4	61.7	61.7	61.2	59.0	52.6	53.5	53.5
	Mean	-	61.6	61.8	63.7	65.4	63.6	62.4	61.3	60.1	60.8	59.7	59.1	55.9	53.0	53.2	54.5
	S.E.	-	1.7	1.8	1.8	1.9	1.8	1.7	1.5	1.3	2.2	1.9	2.3	2.3	1.4	1.9	2.0

Appendix 7. Table 2

Packed Cell Volumes (%), following a challenge infection with a virulent strain of *S. mattheei*, in sheep previously exposed to a non-virulent strain of *S. mattheei* and worm-free sheep

Group	Sheep No.	Weeks After Challenge															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control	55	33	31	30	35	32	32	33	31	32	31	35	31	29	32	31	32
	51	34	35	32	34	36	36	36	33	33	33	35	28	32	34	33	36
	32	32	34	31	33	32	34	32	33	32	31	33	26	30	31	31	34
	8	37	36	36	41	37	38	38	34	36	36	36	36	34	36	33	35
	Mean	34	34	32	36	34	35	35	33	33	33	35	30	31	33	32	34
	S.E.	1	1	1	2	1	1	1	1	1	1	1	2	1	1	1	1
Vaccinates	7	30	29	29	33	31	26	27	28	28	23	23	23	23	23	23	25
	52	30	N.D.	30	32	30	31	30	28	30	30	28	29	26	27	27	25
	60	33	32	32	34	32	31	31	32	32	31	28	25	26	24	22	24
	19	29	31	30	31	29	27	28	29	29	29	29	28	26	27	26	27
	Mean	31	31	30	33	31	29	29	29	30	28	27	26	25	25	25	25
	S.E.	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
Non-Vaccinates	11	34	35	32	35	30	31	30	25	23	25	22	18	19	16	17	15
	95	40	39	42	40	38	38	36	30	31	27	28	26	28	27	26	26
	68	35	37	34	35	31	30	33	23	20	21	20	19	20	20	20	19
	190	36	34	32	33	31	29	28	27	27	25	26	22	21	20	20	20
	Mean	36	36	35	36	33	32	32	26	25	25	24	21	22	21	21	20
	S.E.	1	1	2	1	2	2	2	1	2	1	2	2	2	2	2	2

N.D. Not Determined

Appendix 7. Table 3

Total Serum Protein Concentrations (g/100ml), following a challenge infection with a virulent strain of *S. mattheei*, in sheep previously exposed to a non-virulent strain of *S. mattheei* and worm-free sheep

Group	Sheep No.	Weeks After Challenge															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control	55	6.32	6.17	6.33	6.69	6.71	6.58	6.65	6.56	6.84	6.36	6.43	6.67	6.52	6.49	6.43	6.24
	51	6.01	6.12	5.85	5.96	6.23	6.55	6.25	5.82	5.92	5.88	6.21	6.44	6.47	6.16	6.43	6.43
	32	5.62	5.28	5.47	5.77	5.76	5.69	5.60	5.67	5.75	5.28	5.36	5.65	5.27	5.63	5.90	5.66
	8	6.10	6.00	6.09	6.86	5.96	6.48	6.75	7.11	6.36	6.43	6.58	6.54	6.87	6.56	6.38	6.34
	Mean	6.01	5.89	5.94	6.32	6.17	6.33	6.31	6.29	6.22	5.99	6.15	6.33	6.28	6.21	6.29	6.17
	S.E.	0.15	0.21	0.18	0.27	0.21	0.21	0.26	0.34	0.24	0.27	0.27	0.23	0.35	0.21	0.13	0.17
Vaccinates	7	6.68	6.96	7.25	8.06	8.27	7.78	8.01	7.82	7.76	7.87	7.68	7.62	7.86	7.86	8.11	8.35
	52	6.74	6.62	6.76	7.17	7.77	7.54	7.25	7.64	7.65	7.80	7.65	7.41	7.63	8.21	8.54	8.21
	60	5.94	6.14	6.63	7.45	6.13	6.67	6.40	6.56	6.49	5.86	6.16	6.92	6.98	7.10	7.34	7.10
	19	5.88	6.14	6.25	6.62	6.69	6.79	7.22	6.61	7.08	7.30	7.01	6.98	7.05	6.99	7.01	6.82
	Mean	6.31	6.47	6.72	7.33	7.22	7.20	7.22	7.16	7.25	7.21	7.13	7.23	7.38	7.54	7.75	7.62
	S.E.	0.23	0.20	0.21	0.30	0.49	0.27	0.33	0.33	0.29	0.47	0.36	0.17	0.22	0.30	0.35	0.39
Non-Vaccinates	11	5.96	6.14	6.02	6.05	6.08	6.19	6.00	5.82	5.81	6.53	6.52	6.24	6.72	7.03	7.22	7.08
	95	6.05	6.10	5.88	5.77	5.79	6.38	5.94	5.61	5.60	5.65	5.56	5.65	5.50	7.31	8.30	8.21
	68	6.41	6.26	5.98	6.45	5.99	6.41	6.72	5.19	5.65	5.42	6.45	6.47	6.42	6.28	6.24	6.19
	190	6.44	6.34	6.40	6.73	6.26	6.65	6.58	6.69	6.81	7.06	7.19	7.59	7.76	7.58	7.10	7.13
	Mean	6.22	6.21	6.07	6.25	6.03	6.41	6.31	5.83	5.97	6.17	6.43	6.49	6.60	7.05	7.22	7.15
	S.E.	0.12	0.05	0.11	0.21	0.10	0.09	0.20	0.32	0.28	0.38	0.33	0.41	0.47	0.28	0.42	0.41

Appendix 7. Table 4

Serum Albumin Concentrations (g/100ml), following a challenge infection with a virulent strain of S. mattheei, in sheep previously exposed to a non-virulent strain of S. mattheei and worm-free sheep

Group	Sheep No.	Weeks After Challenge															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control	55	3.63	3.56	3.62	3.73	3.85	3.54	3.69	3.74	3.90	3.42	3.61	3.81	3.75	3.87	3.98	3.98
	51	3.21	3.17	3.23	3.57	3.48	3.22	3.68	3.48	3.61	3.91	3.80	3.71	3.87	3.72	4.00	4.02
	32	3.41	3.34	3.35	3.39	3.31	3.49	3.44	3.36	3.47	3.61	3.43	3.52	3.45	3.54	3.83	3.92
	8	3.47	3.54	3.56	3.61	3.31	3.39	3.34	3.49	3.43	3.44	3.55	3.56	3.64	3.59	3.66	3.93
	Mean	3.43	3.40	3.44	3.58	3.49	3.41	3.54	3.52	3.60	3.60	3.60	3.65	3.68	3.68	3.87	3.96
	S.E.	0.09	0.09	0.09	0.07	0.13	0.07	0.09	0.08	0.11	0.11	0.08	0.07	0.09	0.07	0.08	0.02
Vaccinates	7	3.34	3.48	3.33	3.10	2.77	2.74	2.76	2.79	2.12	2.14	2.00	1.90	2.35	2.54	2.83	2.46
	52	3.65	3.54	3.23	3.03	2.82	3.22	2.70	2.64	2.48	2.57	2.48	2.63	2.47	2.61	2.70	2.97
	60	3.30	3.39	3.36	3.26	3.11	3.61	3.37	3.06	2.80	1.97	2.20	2.38	2.47	2.22	2.13	2.14
	19	2.89	2.97	2.99	3.07	2.98	3.11	3.33	3.20	3.05	2.83	2.84	2.92	2.84	2.81	2.91	2.85
	Mean	3.30	3.35	3.23	3.12	2.92	3.17	3.04	2.92	2.61	2.38	2.38	2.46	2.53	2.55	2.64	2.61
	S.E.	0.16	0.13	0.08	0.05	0.08	0.18	0.18	0.13	0.20	0.20	0.18	0.22	0.11	0.12	0.18	0.19
Non-Vaccinates	11	3.49	3.56	3.41	3.33	3.24	3.49	2.94	2.17	1.72	1.70	1.54	1.24	1.24	1.51	1.70	1.52
	95	3.62	3.73	3.59	3.61	3.63	3.71	3.26	2.36	1.99	1.85	1.71	1.60	1.43	1.91	2.37	2.50
	68	3.50	3.66	3.47	3.26	3.58	3.83	2.90	1.96	1.76	2.00	1.80	1.60	1.72	1.67	1.69	1.69
	190	3.61	3.39	3.43	3.55	3.45	3.51	2.92	2.61	2.70	2.61	2.47	2.54	2.70	2.83	2.97	2.77
	Mean	3.56	3.59	3.48	3.44	3.48	3.64	3.01	2.28	2.04	2.04	1.88	1.75	1.77	1.98	2.18	2.12
	S.E.	0.03	0.07	0.04	0.08	0.09	0.08	0.08	0.14	0.23	0.20	0.20	0.28	0.32	0.29	0.31	0.30

Appendix 7. Table 5

Serum Globulin Concentrations (g/100ml), following infection with a virulent strain of S. mattheei, in sheep previously exposed to a non-virulent strain of S. mattheei and worm-free sheep

Group	Sheep No.	Weeks After Challenge															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control	55	2.69	2.61	2.71	2.96	2.86	3.04	2.96	2.82	2.94	2.94	2.82	2.86	2.77	2.62	2.45	2.26
	51	2.80	2.95	2.62	2.39	2.75	3.33	2.57	2.34	2.31	1.97	2.41	2.73	2.60	2.44	2.43	2.41
	32	2.21	1.94	2.12	2.38	2.45	2.20	2.16	2.31	2.28	1.67	1.93	2.13	1.82	2.09	2.07	1.74
	8	2.63	2.46	2.53	3.25	2.65	3.09	3.41	3.62	2.93	2.99	3.03	2.98	3.23	2.97	2.72	2.41
	Mean	2.58	2.49	2.50	2.75	2.68	2.92	2.78	2.77	2.62	2.39	2.55	2.68	2.61	2.53	2.42	2.21
	S.E.	0.13	0.21	0.13	0.22	0.09	0.25	0.27	0.31	0.18	0.34	0.24	0.19	0.29	0.18	0.13	0.16
Vaccinates	7	3.34	3.48	3.92	4.96	5.50	5.04	5.25	5.03	5.64	5.73	5.68	5.72	5.51	5.32	5.28	5.89
	52	3.09	3.08	3.53	4.14	4.95	4.32	4.55	5.00	5.17	5.23	5.17	4.78	5.16	5.60	5.84	5.24
	60	2.64	2.75	3.27	4.19	3.02	3.06	3.03	3.50	3.69	3.89	3.96	4.54	4.51	4.88	5.21	4.96
	19	2.99	3.17	3.26	3.55	3.71	3.68	3.89	3.41	4.03	4.47	4.17	4.06	4.21	4.18	4.10	3.97
	Mean	3.02	3.12	3.50	4.21	4.30	4.03	4.18	4.24	4.63	4.83	4.75	4.78	4.85	5.00	5.11	5.02
	S.E.	0.14	0.15	0.15	0.29	0.57	0.42	0.47	0.45	0.46	0.41	0.41	0.35	0.30	0.31	0.36	0.40
Non-Vaccinate	11	2.47	2.58	2.61	2.72	2.84	2.70	3.06	3.65	4.09	4.83	4.98	5.00	5.48	5.52	5.52	5.56
	95	2.43	2.37	2.29	2.16	2.16	2.67	2.68	3.25	3.61	3.80	3.85	4.05	4.07	5.40	5.93	5.71
	68	2.91	2.60	2.51	3.19	2.41	2.58	3.82	3.23	3.89	3.42	4.65	4.87	4.70	4.61	4.55	4.50
	190	2.83	2.95	2.97	3.18	2.81	3.14	3.66	4.08	4.11	4.45	4.72	5.05	5.06	4.75	4.13	4.36
	Mean	2.66	2.63	2.60	2.81	2.56	2.77	3.31	3.55	3.93	4.13	4.55	4.74	4.83	5.07	5.03	5.03
	S.E.	0.12	0.12	0.14	0.24	0.16	0.12	0.26	0.20	0.12	0.32	0.24	0.23	0.30	0.23	0.42	0.35