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The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on food intake and utilization in both immunologically naïve and competent sheep

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Abstract

The nutritional cost of both the acquisition and maintenance of immunity to gastro-intestinal nematodes was investigated using immunologically naïve 5-month-old lambs and immunologically competent 17-month-old ewes. Within each age cohort, animals were either infected with the equivalent of 80 L3 Trichostrongylus colubriformis *larvae per kg live weight (LW) per day (IF), similarly infected and concurrently immuno-suppressed with weekly injections of 1·3 mg/kg LW of the glucocorticoid methylprednisolone acetate (ISIF), immuno-suppressed only (IS) or remained as controls (C). Body composition of all animals was estimated using X-ray computer tomography on days -14 and 76 relative to the start of infection. Body weight and faecal nematode egg counts (FEC; eggs per gram of fresh faeces (e.p.g.)) were taken weekly and blood samples for serum proteins and antibodies were obtained every 2 weeks. FEC in IF lambs peaked at 1250 e.p.g. before a typical decline as immunity developed to less than 100 e.p.g. by day 75. FEC of less than 100 e.p.g. in IF ewes indicated immunity was maintained. Successful immuno-suppression in ISIF lambs and ewes was indicated by FEC of 4000 e.p.g. on day 75 and was confirmed by comparative worm burdens and serum antibody titres. The typical reduction in voluntary food intake (VFI) as a consequence of infection was observed in IF lambs (proportionately 0·30,* $P < 0·001$*) but not in IF ewes, ISIF lambs or ISIF ewes. Gross efficiency of use of metabolizable energy for net energy deposition was reduced by proportionately 0·20 in lambs during acquisition of immunity and by 0·16 in ewes maintaining an established immunity. Infection in immuno-suppressed animals reduced efficiency by 0·05 and 0·15 for lambs and ewes, respectively. These findings allowed the hypothesis that the reduction in VFI and loss in performance in young parasitised sheep is caused by physiological signalling associated with the acquisition phase of the host immune response to infection, rather than simply the damage caused by the parasite* per se*.*

Keywords: *energy consumption, food intake, immunity, nematoda, sheep.*

Introduction

The enhancement of natural immune responses of sheep to reduce the metabolic and productive cost of gastro-intestinal parasitism has recently received considerable research attention. Theoretically, given the ubiquitous nature of nematode parasites, host immunity might be anticipated to facilitate productivity and productive genotypes to have stronger immunity than their unselected counterparts. However, attempts to increase the natural immunity by selection of animals maintaining low faecal egg counts (FEC) in the face of larval challenge have not always been associated with the anticipated increase in productivity (Morris *et al*., 2001; Kahn *et al*., 2003). Additionally, sheep selected for production traits such as growth rate and fleece weight have often been observed to have higher FEC than randomly bred animals (Howse *et al*., 1992; McEwan *et al*., 1992). Together, these findings support a hypothesis that immunity has a significant nutritional cost and that the consequent prioritizing of nutrients may compromise productivity (Coop and Kyriazakis, 1999; Colditz, 2002).

Clinical and subclinical intestinal parasitism has been associated with increased endogenous losses of protein into the alimentary tract and increased cost of repair of damage caused by the parasite (MacRae, 1993; van Houtert and Sykes, 1996). In addition, the inevitable reduction in voluntary food intake limits productivity (van Houtert and Sykes, 1996). Despite suggestions that the reduction of intake may carry some functional rôle in limiting the effects of parasitism on the host animal (Kyriazakis *et al*., 1998), neither its function nor mechanisms are understood. In a recent study in our laboratory in which, for other purposes, the immune responses of mature sheep were suppressed with corticosteroids (Nagasinha, 1999), no reduction in intake was observed during infection with *Trichostrongylus colubriformis,* despite the establishment of extremely large

worm burdens (> 260 000). Furthermore, investigations in murine and human subjects have identified the possibility that cytokines involved in cell signalling during the immune response may disrupt feeding behaviour and growth (Johnson, 1997; Spurlock, 1997). It may be hypothesized, therefore, that components of the immune response, rather than simply the damage caused by the parasite *per se*, may be implicated in the reduction in performance of infected sheep. This paper describes the effects of immuno-suppression in immunologically naïve and competent sheep during nematode larval challenge on intake, performance and food utilization.

Material and methods

Animals and treatments

Thirty-six Coopworth ewe lambs were suckled by their dams at pasture before being weaned and housed at three weeks of age, to minimize nematode larval experience, until the start of the trial at 5 months of age. In addition, 36 17-month-old Coopworth ewe hoggets (ewes), which had been subjected to normal farm practices, and were assumed to have developed immunity through natural exposure to nematode larvae, were brought indoors off pasture 2 weeks prior to the initiation of infection. All animals were drenched with 1 ml per 5 kg live weight (LW) of a combination drench (37·5 g/l levamisole and 23·8 g/l albendazole, Arrest, Ancare New Zealand Ltd, Auckland, New Zealand) when removed from pasture. Animals within each age cohort were allocated hierarchically by fasted LW into one of four groups (no. = 9), mean LW 26·6 (s.e. 0·62) and 47·6 (s.e. 1·47) kg for lambs and ewes, respectively. One group of each cohort was infected with the equivalent of 80 L3 *T. colubriformis* larvae per kg LW per day (IF), a second group (ISIF) received the same infection but with immune function suppressed by weekly intramuscular injection of the glucocorticoid methylprednisolone acetate, (Depredone, 40 mg methylprednisolone acetate per ml Jurox Pty. Ltd, Rutherford, NSW, Australia) at a rate of 1 ml per 30 kg LW. This regime had been shown previously by Nagasinha (1999) to be successful in preventing establishment of immunity in sheep without compromising animal health. A third group received only the glucocorticoid (IS) and the fourth remained as a control (C), creating two 2 × 2 factorial designs. The experimental design is given in Table 1.

Feeding and sampling

Animals were offered fresh water and a complete ruminant ration *ad libitum* daily (Table 2). Individual food refusals were collected and weighed weekly. Subsamples of food offered and refused were taken for determination of dry matter (DM) after drying for 72 h at 90°C. Live weight was recorded at weekly intervals. Fasted LW (after 24 h of food deprivation) was also measured on days -7 and 77 of infection to aid the estimation of body composition by computer tomography (described later). Blood samples were taken every two weeks from day -7 using jugular venipuncture into a 10 ml vacutube (Becton Dickinson, VACUTAINER Systems, Rutherford, New Jersey, USA) and immediately placed at 4°C. After centrifugation at 2500 r. p. m. for 20 min serum was separated and stored at -20°C. Rectal temperature was measured every 2 weeks.

Table 1 *Experimental design: 5-month-old lambs and 17-month-old ewes were infected* (*+*)*, or not* (-) *for 78 days with the equivalent of 2000 or 3200 L3* T. colubriformis *larvae per day, respectively, with normal* (-) *or suppressed immunity* (*+*)

Group	No.		Immuno-suppressed	Infection
	Ewes	Lambs		
C	9	9	-	-
IF	9	9	-	+
ISIF	9	9	+	+
IS	9	9	+	-

Table 2 *Diet composition* (*g/kg dry matter* (*DM*)) *and analysis for the complete diets offered* ad libitum *to the 5-month-old lambs and 17-month-old ewes*

	Diet offered	
	Lambs	Ewes
Composition		
Fish meal	100	
Molasses	50	50
Barley	426	100
Lucerne chaff	400	292
Broll		540
Salt	1·75	2
Dibasic calcium phosphate	11	5
Potassium carbonate	8·5	8·5
Magnesium oxide	0·75	0·5
Mineral mix†	2	2
Analysis‡		
CP (g/kg DM)	205	174
ME (MJ/kg DM)	10·1	8·5
DTUP5	33·1	7·4
RP5	130	127

† 2 kg contains: 1·5 g retinol, 0·05 g cholecalciferol, 5·0 g alpha-tocopherol, 1 g Co, 1 g I, 20 g Mn, 26 g Fe, 25 g Zn, 210 g Mg, 4 g P, 0·2 g Se, 560 g Ca.

‡ CP = crude protein; ME = metabolizable energy; DTUP5 = digestible true undegraded protein at 5% per h rumen outflow rate (AFRC, 1993); RP5 = rumen-degradable protein at 5% rumen outflow rate (AFRC, 1993).

Parasitology

Lambs (groups IF and ISIF) were infected with the equivalent of 2000, and ewes with 3200 L3 *T. colubriformis* larvae per day (80 L3 larvae per kg initial LW per day) in three doses each week from day 0 until day 72. The larvae were pipetted from an aqueous solution of known larval content onto moist filter paper, which was then rolled and administered using a balling gun. Faecal samples were taken directly from the rectum of each sheep at weekly intervals from day -6. FEC were measured using a modification of the McMaster method (Ministry of Agriculture, Fisheries and Food, 1979) and expressed as eggs per g of fresh faeces (e.p.g.). Infected lambs and ewes were fasted for 24 h before slaughter on days 77 and 78, respectively. Slaughter and worm recovery in the small intestine was achieved using methods described by Donaldson *et al*. (2001).

Serum analysis

T. colubriformis-specific total antibody and immunoglobulin A (IgA) in the serum were measured using an enzyme-linked immunosorbent assay (ELISA) as described by Xie *et al*. (2004), with the exception that colour was developed for 12 and 40 min for total antibody and IgA, respectively.

Serum urea, albumin and total protein were analysed on a Cobas Mira Plus Auto-analyser (Roche Diagnostics GmbH, Mannheim, Germany) using kits no. 1489364, no. 1970569 and no. 1553836, respectively.

Body composition

Changes in the bone, muscle and fat content of the carcass were estimated *in vivo* using X-ray computer tomography (CT) on days -14 and 76 of infection. Sedation and restraint were as described by Donaldson *et al*. (2001). Scanning procedure was similar to that of Young *et al*. (1996), whereby three anatomical reference X-ray cross sections were taken from each animal at the thoracic vertebrae 8, lumbar vertebrae 5 and ischium. In addition, ten animals (five lambs and five ewes) were randomly selected on each occasion for estimation of total carcass tissue weights using the calvaleri principle of Gunderson *et al*. (1988). Carcass weights (CW) for all animals at both scanning times were estimated using the formula:

$$CW\ (kg) = (0{\cdot}6206 \times \text{fasted LW}) - 3{\cdot}6182$$

that was derived by regressing the actual CW with fasted LW of those slaughtered on day 76 ($R^2 = 0{\cdot}97$). As anticipated, bone represented in the three reference slices did not correlate well with carcass bone found using the calvaleri procedure ($R^2 = 0{\cdot}13$). Consequently bone weight in the carcass was estimated using the following equation derived from Fourie *et al*. (1970):

$$\text{bone wt (kg)} = 0{\cdot}2491 \times CW^{0{\cdot}7321}$$

and then subtracted from the CW to give bone-free CW. Proportions of fat and muscle in the three reference slices did correlate well with proportions of fat and muscle found in the bone-free CW using the calvaleri procedure ($R^2 = 0{\cdot}94$, for both), and were corrected accordingly using the following equations:

calvaleri fat (g per 100 g) = (1·0274 × fat (g per 100 g) in the reference slices) - 0·63;

calvaleri muscle (g per 100 g) = (1·0274 × muscle (g per 100 g) in the reference slices) - 0·0211

to give the proportions of fat and muscle in the bone-free CW. The proportions of fat and muscle were then multiplied by the bone-free CW to give total fat and muscle weights in each individual. The energy deposited in the carcass gain was calculated assuming muscle tissue consisted of proportionately 0·20 protein using energy values of 38·9 and 22·2 MJ/kg for fat and protein, respectively (Blaxter and Rook, 1953).

Wool production

Animals were shorn on days -7 and 77 of infection, and greasy fleece weight recorded at the latter used as a measure of total wool production. A subsample of mid-side fleece wool was taken at shearing from each animal on day 77 and stored at 20°C with 0·65 relative humidity until commercially scoured for estimation of clean fleece weight. Energy deposition in the fleece was calculated assuming 23·7 MJ/kg clean fleece weight (Agricultural and Food Research Council (AFRC), 1993).

Food digestibility

An additional eight male hogget rams were housed in metabolism crates to determine digestibility of the diet offered to the lambs. Once adjusted to the diet, four animals were treated with methylprednisolone acetate as described previously. All animals were offered *ad libitum* access to the diet during the following eight days. Food refusals and faeces production from each animal were weighed and bulked daily, before storage at 4°C. Subsamples of food offered, food refused and faeces were dried to a constant weight at 90°C in a forced air oven. The animals were then adjusted to the diet offered to the ewes and the procedure repeated.

Statistical analysis

Data from lamb and ewe groups were analysed as two separate 2 × 2 factorial experiments using GENSTAT statistical package (Lawes Agricultural Trust, 2001). Faecal egg counts and worm burdens were log transformed ($\log_{10}$ (count + 1)) before analysis. Worm burden, wool production, digestibility and carcass composition were analysed by ANOVA. All remaining measurements underwent sequential comparison of ante-dependence structures for repeated measures before being analysed by REML with estimates of missing values.

This experiment was carried out with approval from, and in accordance with the Lincoln University Animal Ethics Committee:Authority LU39/01.

Results

Of the nine lambs allocated to group ISIF, one (no. 55) was suspected of not responding to the immune-suppressive

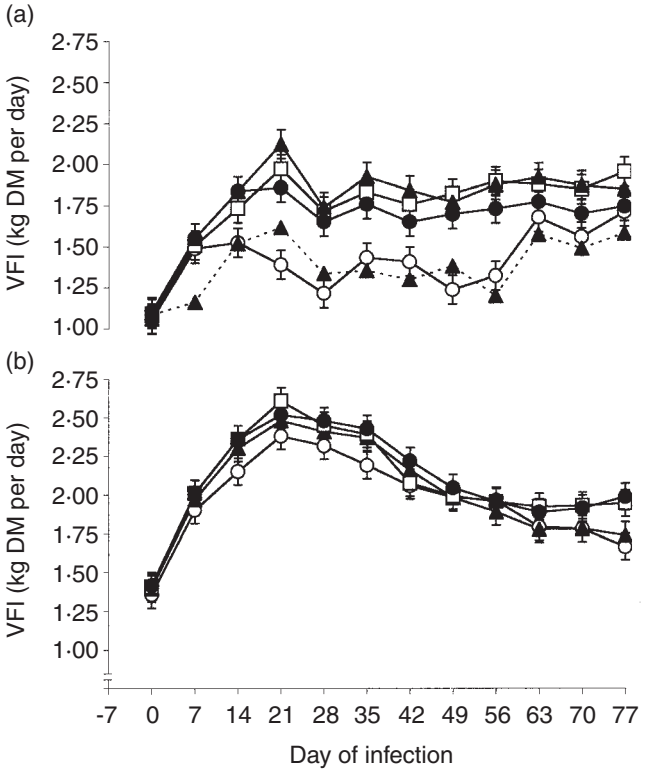

Figure 1 Mean daily voluntary food intakes (VFI, dry-matter (DM) basis) for (a) 5-month-old lambs and (b) 17-month-old ewes: —○— infected with 2000 or 3200 *T. colubriformis* per day (IF), —▲— similarly infected but immune-suppressed (ISIF), —●— immune-suppressed only (IS), —□— controls (C), and - - ▲ - - animal no. 55.

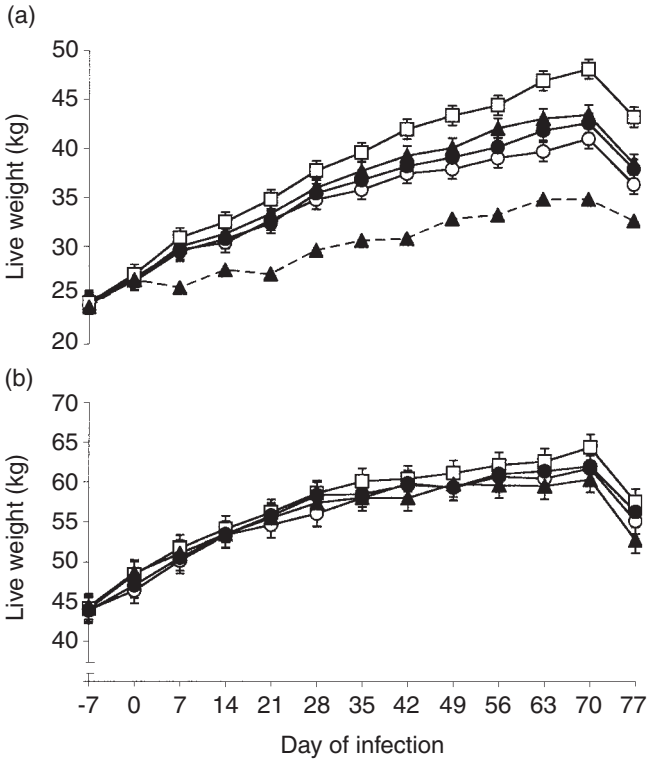

Figure 2 Mean live weights of (a) 5-month-old lambs and (b) 17-month-old ewes: —○— infected with 2000 or 3200 *T. colubriformis* per day (IF), —▲— similarly infected but immune-suppressed (ISIF), —●— immune-suppressed only (IS), —□— controls (C), and - -▲- - animal no. 55.

treatment. Parasite-specific IgA measurements, (Figure 5) indicated incomplete immune-suppression; consequently this individual was excluded from means and statistical analyses, and its data are provided separately. One lamb from group IF died on day 38 and one from group IS on day 74. Post-mortem examination revealed the cause of death to be pneumonia and pulpy kidney, respectively. Data from these animals were included in statistical analysis using missing value estimations. Mean rectal temperature was 38·7°C (s.e. 0·12) for lambs and 38·4°C (s. e. 0·09) for ewes. There was no treatment × time interaction in either group ($P > 0·05$ for both), nor was there any effect of treatment on rectal temperature. Faecal DM was not measured, however it was observed that no ISIF lamb or ewe had softened faeces or displayed any clinical signs of parasitism at any stage.

Food intake and digestibility

Food intake of lambs and ewes is shown in Figure 1. For the lambs, there was a significant treatment × time interaction ($P < 0·01$) reflecting a 0·30 reduction in intake of IF lambs from day 21 of infection ($P < 0·001$), before recovery by day 63. In contrast, intake of ISIF lambs was similar to that of C and IS groups ($P > 0·05$). Sheep no. 55 from the ISIF lambs displayed a similar pattern of intake to that of IF lambs (Figure 1). There were no treatment × time interactions for DM intake in ewes, nor were mean DM intakes different, being 2·01, 2·10, 1·96 and 2·09 kg DM per day for ISIF, IS, IF and C ewes, respectively ($P > 0·05$). However, temporary proportional reductions of 0·09 ($P < 0·05$) and 0·14 ($P < 0·05$) were observed in IF compared with C ewes on days 21 and 77, respectively.

DM digestibility was not affected by immuno-suppression, being 0·68 and 0·58 for the diets offered to lambs and ewes, respectively.

Live weight

Changes in mean LW of lambs and ewes are given in Figure 2. There was a significant treatment × time interaction amongst groups of lambs ($P < 0·001$). Lambs in group IF were significantly lighter than group C ($P < 0·05$) at day 35, a difference which increased progressively until day 77 when IF had proportionately 0·16 lighter fasted LW ($P < 0·001$). LW gain was lower in uninfected immuno-suppressed lambs, with group IS significantly lighter than group C from day 49 onwards ($P < 0·05$), and proportionately 0·12 lighter on day 77 ($P < 0·01$). Live weight of ISIF lambs was at all times comparable with, and not significantly different from that of IS ($P > 0·05$). Impairment of growth due to infection was prevented by immune-suppression, with ISIF lambs being proportionately 0·09 heavier than IF lambs between days 56 and 70 of infection ($P < 0·05$). In ewe groups, there was no treatment × time interaction ($P > 0·05$), though ISIF ewes did have an 0·08 proportionately lighter fasted LW than C ewes on day 77 ($P < 0·05$).

Faecal egg counts and worm burdens

The mean FEC of lambs and ewes are shown in Figure 3. Eggs were detected from day 20 of infection in IF lambs, and peaked at 1250 e.p.g. on day 41 before declining to less than 100 e.p.g. by day 75. Lambs in group ISIF showed a similar trend in FEC to group IF during the first 41 days of infection but thereafter FEC continued to rise and were greater than IF (plateau of about 4000 e.p.g.) on day 62 ($P < 0·001$). Eggs were not detected in uninfected groups of lambs. Eggs were not observed in the faeces of IF ewes until day 41 of infection.

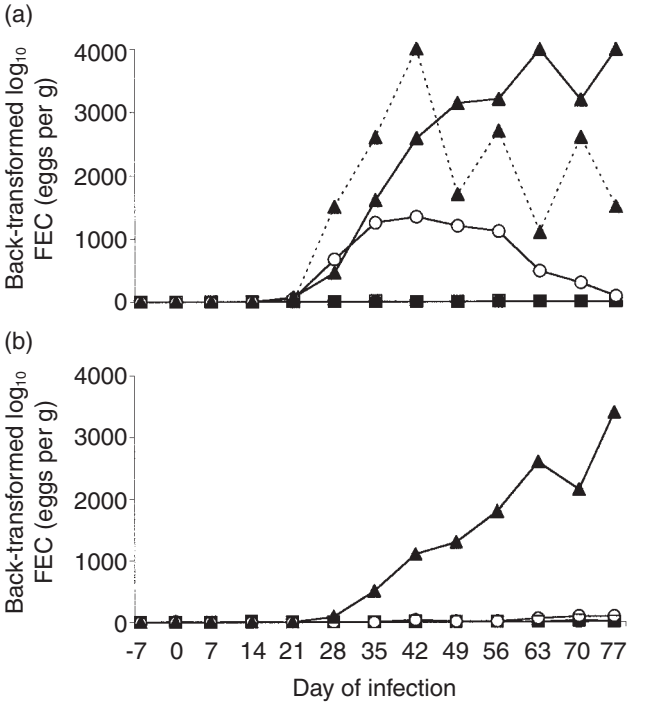

Figure 3 Mean back-transformed $\log_{10}$ faecal egg count (FEC) of (a) 5-month-old lambs and (b) 17-month-old ewes: —○— infected with 2000 or 3200 *T. colubriformis* per day (IF), —▲— similarly infected but immune-suppressed (ISIF), —●— immune-suppressed only (IS), —□— controls (C), and ---▲--- animal no. 55.

Table 3 *Numbers of log_{10} (count + 1) worms recovered from 5-month-old lambs and 17-month-old ewes infected with 2000 and 3200 L3* T. colubriformis *larvae per day, respectively, for 77 days and which had an intact immunity* (*IF*) *or were immune-suppressed* (*ISIF*) (*back-transformed values are given in parenthesis*)

	5-month-old lambs			17-month-old ewes		
Larval development	ISIF	IF	s.e.	ISIF	IF	s.e.
L3	0	0		0·37[a] (2)	1·00[a] (10)	0·19
L4	0	0		2·71[a] (512)	0·39[b] (2)	0·19
L5	4·35[a] (22387)	2·35[b] (224)	0·46	4·40[a] (25118)	2·17[b] (148)	0·19
Total	4·35[a] (22387)	2·35[b] (224)	0·46	4·41[a] (25632)	2·20[b] (160)	0·26

[a,b] Within each age cohort and each stage of larval development, values with different superscripts are significantly different ($P < 0·01$).

Counts of less than 100 e.p.g. were then maintained until the conclusion of the trial. In contrast, the mean FEC in ISIF ewes was significantly greater than IF from day 27 onwards ($P < 0·01$), with counts approaching 4000 e.p.g. by the conclusion of the trial. Eggs were not found in uninfected ewes.

Back-transformed geometric mean worm burdens are shown in Table 3. Mean worm burdens of 22 387 and 224 in ISIF and IF lambs, respectively, differed significantly ($P < 0·01$). Burdens in both lamb treatments were made up entirely of L5 adult worms. Similar numbers of worms were recovered from infected ewes, mean values being 25 632 and 160 for ISIF and IF groups, respectively, ($P < 0·001$). Low numbers of L3 and L4 worms were found in infected ewes, with adult worms contributing 0·98 and 0·91 of total burden in ISIF and IF groups, respectively. Numbers of L4 worms recovered were low but significantly different between ISIF and IF ewes ($P < 0·001$), being 512 and 2 larvae, respectively.

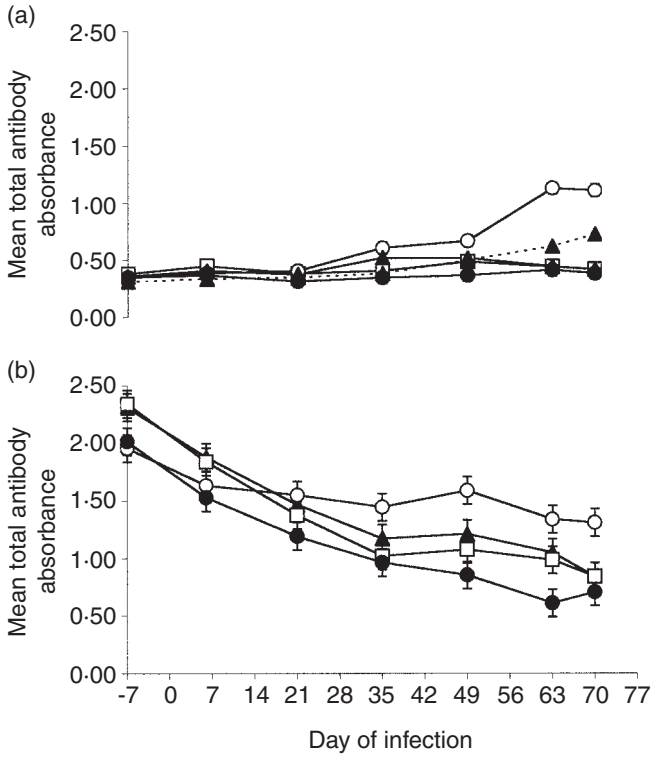

Figure 4 Mean total parasite-specific L3 *T. colubriformis* antibody levels of (a) 5-month-old lambs and (b) 17-month-old ewes: —○— infected with 2000 or 3200 *T. colubriformis* per day (IF), —▲— similarly infected but immune-suppressed (ISIF), —●— immune-suppressed only (IS), —□— controls (C), and ···▲··· animal no. 55.

Serum analyses

Absorbance of total L3 antibody to *T. colubriformis* in lambs and ewes during infection is given in Figure 4. In lambs there was a significant group × time interaction ($P < 0·001$). Levels in IF lambs increased gradually from day 21 to a three-fold increase by day 63 and thereafter ($P < 0·001$). Antibody levels in the three remaining groups of lambs were similar and showed no change during the trial. In the ewes, there was a significant treatment × time interaction ($P < 0·001$) and an effect of time ($P < 0·001$). These reflected the fact that antibody levels declined continuously with time, but at a slower rate in IF and at a greater rate in IS ewes, which were immuno-suppressed without exposure to nematode larvae.

Mean L3 parasite-specific IgA absorbance for lamb and ewe groups is shown in Figure 5. There was no treatment × time interaction amongst lamb groups ($P > 0·05$). However, there was an effect of infection ($P < 0·01$) and time ($P < 0·01$). A rise in mean absorbance in IF lambs peaked at approximately three times that of C lambs on day 49 ($P < 0·01$) before declining to levels 2·2 times higher at day 63 ($P < 0·05$). IgA levels in C, IS and ISIF lambs were similar and showed no change with time. Lamb no. 55 expressed an IgA profile similar to that of IF lambs. In all four ewe groups mean IgA absorbance was low throughout the trial, with levels similar to those in the uninfected lambs. Statistically there was a time × treatment interaction ($P < 0·001$). However, there was no consistent pattern between groups and the differences were extremely small, and not considered to be biologically important.

Changes in serum urea concentrations in lamb and ewe groups are given in Figure 6. In lambs there was a significant effect of time ($P < 0·001$) and of immuno-suppression ($P < 0·001$) but no treatment × time interaction ($P > 0·05$). Urea concentrations increased in all groups with time. Serum urea concentrations in ISIF and IS lambs were consistently

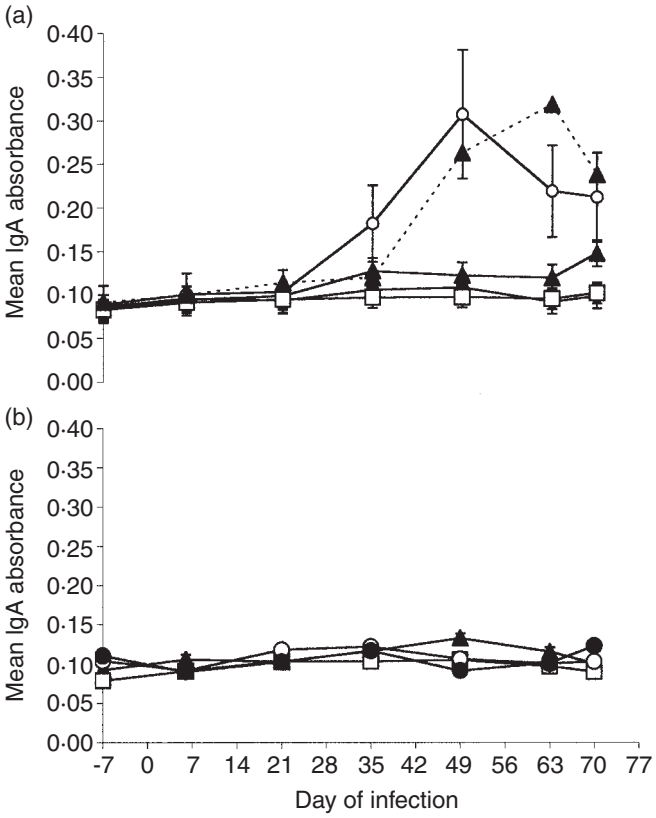

Figure 5 Mean parasite-specific L3 *T. colubriformis* IgA levels of (a) 5-month-old lambs and (b) 17-month-old ewes that were —○— infected with 2000 or 3200 *T. colubriformis* per day (IF), —▲— similarly infected but immune-suppressed (ISIF), —●— immune-suppressed only (IS), —□— controls (C), and --▲-- animal no. 55.

elevated compared with IF lambs throughout, being proportionately 0·14 to 0·31 greater from day 49 ($P < 0·05$) but were only significantly greater than in C lambs on days 49 and 70 ($P < 0·05$ on both occasions). In ewe groups there was a significant effect of time ($P < 0·001$), infection ($P < 0·05$) and immuno-suppression ($P < 0·001$), but no interaction between treatment and time ($P > 0·05$). These effects were reflected in the increase in values with time in all animals, and greater levels in immuno-suppressed animals. Within immuno-suppression treatments, urea values tended to be greater in those exposed to nematode larvae, although this was only significant for ISIF and IF ewes relative to IS and C, respectively, on day 49 ($P < 0·05$ in both cases).

Changes in serum albumin concentrations for lamb and ewe groups are given in Figure 7. In lamb groups, there was a significant treatment × time interaction ($P < 0·001$). This reflected a significant decline in concentrations in IF lambs during the course of infection, while the remaining groups suffered only a temporary reduction between days 21 and 49. In ewe groups, there was a significant effect of immuno-suppression ($P < 0·001$) and time ($P < 0·001$) but no interaction between treatment and time ($P > 0·05$). Mean serum albumin increased with time and tended to be greatest in immuno-suppressed animals. In contrast to lamb groups, infected (IF) ewes did not show a reduction in serum albumin. Serum total protein concentrations were not affected by treatment in either lambs or ewes, mean values being 61·6 (s.e. 1·1) and 65·7 (s.e. 1·2) g/l, respectively.

Carcass composition and wool growth

Computer tomographic estimates of carcass change and estimates of wool growth are shown in Table 4. Amongst groups of lambs there was a significant immuno-suppression × infection interaction for the amounts of fat ($P < 0·01$), muscle ($P < 0·05$) and bone ($P < 0·001$) deposited in the carcass. Infection alone (IF lambs) reduced fat deposition by proportionately 0·38 ($P < 0·05$) and muscle growth by proportionately 0·31 ($P < 0·05$). Irrespective of whether infected or not, immuno-suppressed lambs (IS and ISIF) had similar but non-significant reductions in fat deposition of proportionately 0·06 and 0·09, respectively, ($P > 0·05$ for both) but large proportional reductions in muscle deposition of 0·73 and 0·54, respectively ($P < 0·05$ in both cases) compared with C lambs. There was no interaction between immuno-suppression and infection amongst ewe groups for fat ($P > 0·05$), muscle ($P > 0·05$) or bone ($P > 0·05$) deposition. Amongst groups of ewes, carcass fat deposition was increased by immuno-suppression in groups IS and ISIF relative to C by proportionately 0·45 ($P < 0·05$) and 0·18 ($P > 0·05$), respectively, and was reduced by 0·23 ($P < 0·05$) by infection alone (IF). Infection superimposed on immune suppression in ewe groups (ISIF) reduced fat deposition by proportionately 0·19 ($P < 0·05$) compared with immuno-suppression alone (IS). Muscle deposition of ewes was reduced by proportionately 0·06 ($P > 0·05$) through infection alone in IF, and by proportionately 0·70 ($P < 0·05$) by immuno-suppression alone (IS). In contrast to the observations in lambs, infection in addition to immune suppression in ewes caused a further reduction ($P < 0·05$) in muscle deposition (ISIF compared with IS).

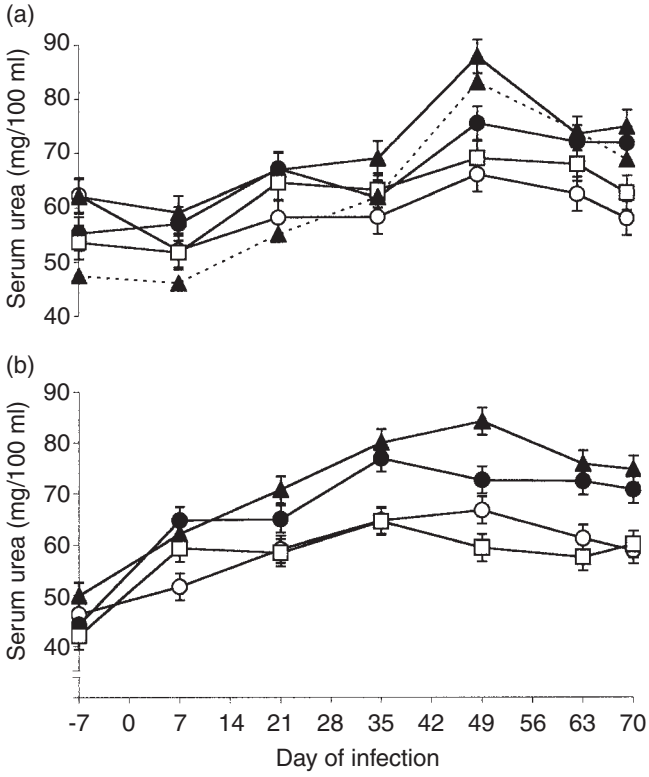

Figure 6 Mean serum urea concentrations of (a) 5-month-old lambs and (b) 17-month-old ewes: —○— infected with 2000 or 3200 *T. colubriformis* per day (IF), —▲— similarly infected but immune-suppressed (ISIF), —●— immune-suppressed only (IS), —□— controls (C), and ---▲-- animal no. 55.

There was an immuno-suppression × infection interaction for wool production in lambs ($P < 0{\cdot}05$) but not ewes ($P > 0{\cdot}05$). Relative to C, clean wool production was significantly reduced by proportionately 0·16, 0·17 and 0·16 in IF, ISIF and IS lambs, respectively, ($P < 0{\cdot}05$ in all cases). Wool production was not affected by infection in ewes, but was reduced by proportionately 0·32 in IS compared with C ewes ($P < 0{\cdot}05$). Infection in addition to immune suppression in ewes caused no further reduction in wool production.

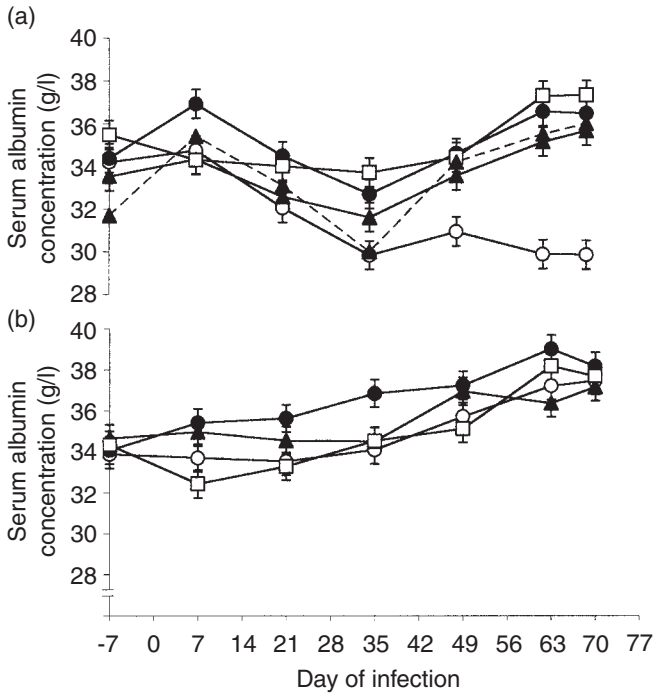

Figure 7 Mean serum albumin concentrations of (a) 5-month-old lambs and (b) 17-month-old ewes: –○– infected with 2000 or 3200 *T. colubriformis* per day (IF), –▲– similarly infected but immune-suppressed (ISIF), –●– immune-suppressed only (IS), –□– controls (C), and ---▲- animal no. 55.

Liver weights of ISIF lambs at slaughter were heavier than IF lambs (899 and 798 (s.e. 33·4) g, respectively, $P < 0{\cdot}05$) but were not different in ewe groups (823 and 915 (s.e. 47·1) g for IF and ISIF ewes, respectively $P > 0{\cdot}05$).

Discussion

These findings provide strong support for the hypothesis that a significant part of the reduction in animal performance during nematode parasitic infection is as a consequence of the host immune response to the parasite rather than simply the effect of damage to the alimentary tract and the cost of its repair, as has been hypothesized in the past (MacRae, 1993; van Houtert and Sykes, 1996). Moreover, the findings disclose the possibility that, in young animals, physiological changes accompanying the acquisition phase of the immune response are implicated in the reduction in appetite that is typical in subclinical infections in young animals with this parasite (Sykes and Coop, 1976; Kyriazakis *et al*., 1996). They also provide novel evidence that the immune response of a mature resistant animal imposes a significant nutritional cost.

The suppression of immunity through the use of corticosteroids was, of necessity, a crude approach, and disturbances to intake, wool growth, live-weight gain, nutrient partitioning and protein metabolism as well as to the immune response were anticipated (Thompson *et al*., 1995; Bertozzi *et al*., 2000), and are discussed later.

The worm burdens of 22 000 and 25 000 in immuno-suppressed (ISIF) lambs and ewes respectively, were much lower than the 267 000 found after immuno-suppression of 15-month-old hoggets by Nagasinha (1999) and low even compared with the 45 000 to 51 000 found by Sykes and Coop (1976) and Bown *et al.* (1991) using similar rates of infection with this nematode in naïve but immunologically normal lambs. The larvae used in the current study can therefore be

Table 4 *Computer tomographically estimated carcass growth, wool production and energy utilization of 5-month-old lambs and 17-month-old ewes: infected with 2000 and 3200 L3* T. colubriformis *larvae per day, respectively,* (*IF*)*, similarly infected and immune-suppressed* (*ISIF*)*, immune-suppressed only* (*IS*)*, control* (*C*)*, and animal no. 55.*

	5-month-old lambs						17-month-old ewes				
	IF	ISIF	IS	C	No. 55	s.e.	IF	ISIF	IS	C	s.e.
Original composition (day -14)											
Live weight (kg)	23·9	24·1	24·2	24·3	23·8	0·64	43·9	44·3	43·0	44·1	1·3
Carcass weight (kg)	11·2	11·3	11·4	11·4	11·2	0·40	23·6	23·9	23·1	23·7	0·8
Bone weight (kg)	1·47	1·47	1·49	1·48	1·46	0·04	2·52	2·54	2·48	2·53	0·06
Fat weight (kg)	1·99	1·88	1·92	1·71	2·20	0·15	7·77	7·36	6·97	7·37	0·41
Muscle weight (kg)	7·86	7·96	8·16	8·25	7·50	0·28	13·3	13·9	13·6	13·8	0·5
Tissue deposition (day 76)											
Bone (kg)	$0{\cdot}67^{a}$	$0{\cdot}78^{b}$	$0{\cdot}74^{ab}$	$1{\cdot}01^{c}$	0·49	0·04	$0{\cdot}52^{b}$	$0{\cdot}40^{a}$	$0{\cdot}62^{b}$	$0{\cdot}62^{b}$	0·05
Fat (kg)	$4{\cdot}41^{a}$	$6{\cdot}46^{b}$	$6{\cdot}63^{b}$	$7{\cdot}08^{b}$	4·12	0·39	$3{\cdot}52^{a}$	$5{\cdot}40^{b}$	$6{\cdot}66^{c}$	$4{\cdot}59^{b}$	0·39
Muscle (kg)	$2{\cdot}54^{b}$	$1{\cdot}70^{ab}$	$0{\cdot}99^{a}$	$3{\cdot}68^{c}$	0·85	0·43	$2{\cdot}89^{c}$	$-0{\cdot}59^{a}$	$0{\cdot}92^{b}$	$3{\cdot}08^{c}$	0·44
Clean wool weight (kg)	$1{\cdot}07^{a}$	$1{\cdot}06^{a}$	$1{\cdot}08^{a}$	$1{\cdot}28^{b}$	0·75	0·04	$1{\cdot}27^{b}$	$0{\cdot}84^{a}$	$0{\cdot}90^{a}$	$1{\cdot}33^{b}$	0·05
Energy utilization†											
Total ME intake (MJ)	1203^{a}	1510^{b}	1485^{b}	1485^{b}	1172	45·7	1418	1460	1518	1509	45·2
Total NE deposited (MJ)	225^{a}	304^{b}	347^{b}	347^{b}	195	15·1	195^{a}	241^{b}	302^{c}	241^{b}	16·4
NE: ME	$0{\cdot}18^{a}$	$0{\cdot}20^{ab}$	$0{\cdot}21^{bc}$	$0{\cdot}23^{c}$	0·16	0·01	$0{\cdot}13^{a}$	$0{\cdot}16^{b}$	$0{\cdot}19^{c}$	$0{\cdot}15^{b}$	0·01

[a,b,c] Within each age cohort and each row, values within with different superscripts are significantly different ($P < 0{\cdot}05$).
† ME = metabolizable energy. NE = net energy.

considered, on this basis, to have had low infectivity. On the other hand, the 0·30 proportional depression in live-weight gain of the infected but non-immuno-suppressed lambs (IF) was comparable with the typical 0·18 to 0·60 proportional reductions observed by Sykes and Coop (1976) and Bown *et al*. (1991) in naïve lambs exposed to similar rates of infection with this parasite, suggesting 'normal' pathogenicity.

The reduction in wool growth in immuno-suppressed lambs and ewes (IS) was expected since glucocorticoid analogues are known to reduce wool production by up to proportionately 0·80 and cause wool breaks that may result in defleecing (Panaretto, 1979). Perhaps surprisingly, therefore, no obvious signs of wool break were observed in any of the immuno-suppressed animals.

Gastro-intestinal nematode infections are known to disturb wool growth (MacRae, 1993). However, larval challenge in ewe groups with a competent immune response did not reduce wool production, findings that contrast with the 0·11 proportional reduction in wool growth in animals resistant to *T. colubriformis* observed by Barger and Southcott (1975). The 0·16 proportional reduction in wool growth in the IF lambs is in agreement with observations in lambs exposed to similar rates of infection by Sykes and Coop (1976). This, together with a lack of effect in immunologically competent ewes suggests competition for potentially rate limiting amino acids for wool production, probably the sulphur amino acids (MacRae, 1993), may be greatest during the phase of acquisition of immunity. Further evidence to support this hypothesis can be observed in data from breeding ewes in which established immunity is temporarily lost in the period around parturition. Leyva *et al*. (1982) infected groups of sheep with *Ostertagia circumcincta* during the last 6 weeks of pregnancy and the first 6 weeks of lactation. These authors observed significant reduction in wool growth only during the latter period, as immunity was being re-established following its relaxation around parturition.

Regardless of infection status, live-weight gain was depressed in young animals treated with the corticosteroid. While some of this reduction in growth rate of the IS group of lambs can be attributed to the 0·03 proportional reduction in food intake, a major cause was the greater than 0·50 proportional reduction in muscle (protein) deposition as a result of immuno-suppression treatment, presumably as a consequence of the net catabolic actions of corticosteroid compounds (Huang *et al*., 1998; Turini *et al*., 2003). It was perhaps surprising, therefore, that a greater than 0·03 reduction in food intake did not occur in IS lambs since demand for energy for protein synthesis has been considered to be a major driver of appetite (Radcliffe and Webster, 1976). The situation created by corticosteroid treatment, therefore, seems to have been one of reduced net muscle protein deposition, presumably resulting in an increased amino acid supply to support the synthesis of constitutive and export proteins in the liver, including acute phase proteins for the immune system (Husband and Bryden, 1996; Calder and Newsholme, 2002). Turini *et al*. (2003) observed in rats treated with dexamethasone that protein synthesis rates were reduced in muscle and increased in the liver, resulting in an increase in liver weight. Evidence for such an effect in the current trial can be seen in the absence of the typical depression in serum albumin and correspondingly greater liver weights of ISIF lambs, and a similar trend in liver weights in ISIF ewes at slaughter compared with their IF counterparts. The relatively high rate of adipose tissue deposition in corticosteroid treated animals has been observed previously in cattle (Dicke *et al*., 1975; Corah *et al*., 1995), and probably reflects an increased availability of deaminated carbon skeletons, as evidenced in elevated serum urea concentrations in immuno-suppressed groups, and presumably low demand for metabolizable energy (ME) for body protein synthesis.

Immuno-suppression appeared to reduce the energetic costs associated with nematode infection. The lowest rate of fat deposition occurred in infected (IF) sheep in both age cohorts (Table 4). Such changes have been interpreted to reflect the energy cost of increased protein synthesis, presumably for the repair of damaged tissue in the alimentary tract (van Houtert and Sykes, 1996; Yu *et al*., 2000). It is interesting, therefore, that animals from both age cohorts, when concurrently infected and immuno-suppressed (ISIF), had rates of adipose tissue deposition that were similar to those of their immuno-suppressed controls (IS) but greater than those of groups simply infected (IF). This suggests that very little energy was invested in the repair of gastro-intestinal tissue in infected immuno-suppressed animals (ISIF). It seems unlikely this can be attributed to differences in ME supply, as the intake of the older (ewe) groups did not differ and we were unable to measure an effect of immuno-suppression on DM digestibility, nor does the literature suggest DM digestibility is likely to be significantly affected by nematode infection (Sykes and Coop, 1976; Bown *et al.,* 1991). Corticosteroids have been shown to cause a reduction in the fractional synthetic rate of total protein in the gut mucosa in non-parasitised rats (Turini *et al*., 2003) which may prevent the restoration of damaged tissue. However, it remains a point of interest that ISIF animals were able to harbour a larger parasite burden than their IF counterparts without displaying any clinical signs of parasitism or suffering a reduction in performance or decline in serum albumin typical of this type of infection. It can be hypothesized, therefore, that the parasite itself caused little direct damage to the intestinal endothelium in ISIF animals. Hein *et al*. (2001) has suggested many of the pathological changes in the gastro-intestinal tract may be undesirable consequences of the immune response. This is supported by the findings of Lawrence *et al*. (2001), in which mast cell deficient mice had longer intestinal villi than their immunologically competent counterparts after 13 days of infection with *T. spiralis*, despite harbouring a greater worm burden. It was hypothesized by Lawrence *et al*. (2001) that one of the rôles of the immune response was to act directly on the gastro-intestinal tissue, making the environment unfavourable for the parasite and, as a consequence, becoming the major cause of intestinal pathology.

The experimental design allowed the comparison of the energetic costs of nematode infection during the development of immunity in young naïve animals (IF lambs) with those incurred by older animals (IF ewes) capable of maintaining a competent immune response as judged by their ability to limit

FEC during larval challenge. The gross efficiency of utilization of ME for growth was calculated as the proportion of total ME intake that was deposited as net energy (NE) in the carcass and fleece (Table 4). Infection alone caused a proportional reduction in the gross efficiency of utilization of ME of 0·20 and 0·16 in IF lambs and ewes, respectively. The reduction in efficiency observed in the lambs is low in comparison to the 0·50 proportional reduction observed in growing lambs with similar rates of infection with this nematode by Sykes and Coop (1976). There are no precise data for the effect of infection on efficiency of ME use in animals with a competent immune response. However, the 0·16 proportional reduction recorded in IF ewes suggests a significant loss in productivity as a consequence of maintaining an established immunity, a figure comparable with the estimated 0·15 proportional increase in maintenance requirement suggested by Sykes (1994).

Reduction in intake during nematode infections of young sheep can vary from less than 0·10 to complete anorexia, and has been calculated to be responsible for 0·60 to 0·90 of the loss in performance during nematode infection (Coop and Holmes, 1996; van Houtert and Sykes, 1996), making it the single largest cause of reduced productivity in nematode-infected sheep. Maintenance of an effective immune response in IF ewes was associated with a non-significant 0·06 proportional reduction in overall intake. There are few reliable data on the effect of parasitic infection on the food intake of immune animals, however intake has often been observed to recover once naïve animals have developed an immunity (Kyriazakis, 1996; Kyriazakis *et al.,* 1998). The reduction in intake exhibited by IF lambs was temporary (Figure 1), occurring only during the phase of acquisition of immunity, between days 14 and 64 of infection. This reduction and subsequent recovery was typical, both temporally and in magnitude, to comparable subclinical infections with this parasite (Kimambo, 1988; Kyriazakis *et al.*, 1996). Surprisingly, neither of the immuno-suppressed and infected groups (ISIF lambs and ewes) suffered a reduction in appetite, despite faecal egg counts approaching 4000 e.p.g. by the end of the trial. The effects of corticosteroids on intake in sheep are not well documented, and appear to vary depending on the dose given. Adams and Sanders (1992) found a short term increase in intake in sheep treated with a single dose of 0·1 mg/kg dexamethasone (equivalent to 0·5 mg/kg methylprednisolone). On the other hand, Panaretto (1979) observed a short term decrease in intake in sheep treated with a larger single dose of 6 mg/kg dexamethasone. We observed no effect of a weekly chronic dose of 1·3 mg/kg methylprednisolone acetate on intake in the current study as at all times both age groups of IS animals consumed food at the same rates as their respective C groups. The results from the current trial provide strong evidence that the greatest challenge to maintenance of food intake during nematode larval challenge occurs during the phase of acquisition of immunity in the naïve lamb. Kyriazakis *et al.* (1998) suggested that anorexia during parasitic infection may be viewed as a disease coping strategy that aids the recognition of the parasite by the immune system, and that strategies to complement it should be considered to allow greater resilience to the effects of infection. While it is still undetermined if anorexia does promote a beneficial immune response, the absence of both anorexia and loss of performance in ISIF lambs suggests a depression in feeding behaviour during nematode infection is not essential for the animal to express greater resilience to cope with infection.

The loss of appetite in IF lambs appeared to coincide with the period of elevation of serum IgA antibodies against *T. colubriformis* L3 larvae. Moreover, food intake of the one lamb from group ISIF (no. 55) which, on the basis of a comparable change in serum IgA to IF lambs (Figure 5) could be considered not to have responded to immuno-suppression, was depressed in a similar pattern to that of IF lambs. Moreover, in the ewes which did not show significant reductions in appetite no elevation of IgA was observed. These data suggest that physiological changes associated specifically with the acquisition phase of the developing immune response and which are associated with the stimulation of the IgA antibody response, rather than the mature immune response *per se*, are responsible for the loss of appetite in infected sheep. A similar relationship between food intake and a component of the immune response was observed in infected lambs by Kimambo *et al.* (1988), whereby elevated eosinophil concentrations during the acquisition phase of the immune response corresponded with the period of maximum reduction in voluntary intake. Furthermore, these authors observed that once immunity was successfully achieved, as judged by faecal egg counts, a decline in eosinophil concentrations was accompanied by an increase in voluntary food intake. Pro-inflammatory cytokines involved in the signalling of the non-specific acute phase response (APR) are considered to act on the hypothalamus through the afferent pathway to cause anorexia, lethargy and thermogenesis in human and murine subjects (Johnson, 1997). While we were unable to detect any increase in rectal temperature in IF animals associated with anorexia, recent reviews have identified IFNγ, TNFα, IL-1 IL-6 and IL-8 as appetite depressing cytokines (Langhans, 2000; Farthing and Ballinger, 2001). We suggest therefore, that the lack of effect on appetite in the immuno-suppressed young lambs resulted from their inability to promote an acute phase cytokine response. In addition, the lack of reduction in appetite in mature ewes reflects the fact that their immune response had evolved to a specific immunity and was therefore less dependent on an APR and the consequences of its cytokine profile.

The evidence suggesting that the immune response *per se* may have detrimental components substantiates reservations raised by Colditz (2002) concerning the benefits of strong immune responses to nematode infection in young lambs. Genetic selection of sheep for low immune responsiveness (high FEC) has resulted in greater productivity in terms of wool growth and live-weight gain, whereas lines selected for low FEC have shown lower growth rates than both their random-bred controls and high FEC lines (Howse *et al.,* 1992; Morris *et al.*, 2001). Pernanther *et al.* (1997) found that *T. colubriformis*-specific antigen stimulation of mesenteric lymph node cells taken from genetically resistant lambs promoted an almost four-fold greater increase in the pro-inflammatory cytokine IFN-γ than cells taken from susceptible lambs. It remains unclear if the reduction in performance in lines selected for a strong immune response is due to greater partitioning of nutrients toward immune

function as suggested by Coop and Kyriazakis (1999), or if a strong developing immune response has physiological consequences that result in a more severe depression of appetite and consequent reduction in performance during the acquisition phase of immunity.

The implications of this work are that a component of the developing immune response, possibly involving pro-inflammatory cytokines, may be responsible for the reduction in voluntary food intake in parasitized lambs. This provides a conflicting situation in which productivity is severely reduced through a reduction in intake during the acquisition phase of immunity but is little affected once an effective immune response is established. The challenge is to identify the exact mechanisms involved and develop strategies that assist the animal in achieving a mature immune status at minimum nutritional cost, or facilitate a rapid shift from the phase of development to the mature immune response.

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