The effect of dietary sainfoin (*Onobrychis viciifolia*) on local cellular responses to *Trichostrongylus colubriformis* in sheep

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SUMMARY

The effect of sainfoin (*Onobrychis viciifolia*) hay consumption on the pathophysiology and local cellular responses of growing lambs during infection with *Trichostrongylus colubriformis* was investigated. Thirty-two lambs, 16 weeks of age, were allocated to 1 of 4 treatment groups ($n=8$) that were offered either grass (G) or sainfoin (S) hay while concurrently either infected (+), or not (−) with 12 000 L3 *T. colubriformis* larvae per week for 6 weeks. Liveweight gains were affected by diet ($P=0{\cdot}002$) and reduced by infection ($P<0{\cdot}005$). Faecal egg count was reduced in S+ compared to G+ from days 35 to 42 ($P=0{\cdot}001$); however, total egg output, worm burdens at day 42 and worm fecundity were similar between diets ($P>0{\cdot}05$). Feeding sainfoin appeared to enhance immune cell development with tissue eosinophils, mast cells and pan T cells present in greater concentrations in S+ than in G+ animals. However, further studies are required to determine if the enhanced immune cell development is a consequence of a greater nutrient supply or a direct influence of sainfoin metabolites on local inflammatory responses to the gastrointestinal nematode *T. colubriformis*.

Key words: sainfoin, *Onobrychis viciifolia*, *Trichostrongylus colubriformis*, plant secondary metabolites (PSM), local immune response, nematodes, sheep.

INTRODUCTION

The prospect of exploiting the potential anti-parasitic effects of plants that contain plant secondary metabolites (PSM) in order to provide an alternative to chemical prophylactic treatments in grazing livestock has stimulated research in this area (Ramirez-Restrepo and Barry, 2005; Hoste *et al*. 2006; Waghorn, 2007). While the identification of plants with anti-parasitic properties has been possible, their incorporation into grazing systems has frequently been restricted due to agronomical issues. Sainfoin (*Onobrychis viciifolia*) is a legume that contains a variety of PSMs such as phenolic glucoside compounds, flavonols, flavonol glycosides and a mixture of procyanidin- and prodelphinidin-type condensed tannins (Marais *et al*. 2000). Furthermore, sainfoins ability to withstand grazing (Lane and Koivisto, 2005) makes it an ideal candidate to provide a therapeutic anti-parasitic effect to infected livestock.

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A number of studies have investigated the anti-parasitic effects of sainfoin for ruminant nematodes both *in vitro* (Paolini *et al*. 2004; Barrau *et al*. 2005) and *in vivo* (Paolini *et al*. 2003*a*, *b*, 2005*a*, *b*). In general, positive anti-parasitic effects have been achieved on intestinal worms where sainfoin consumption resulted in lower faecal egg counts and total egg output (Athanasiadou *et al*. 2005; Paolini *et al*. 2003*a*, *b*, 2005*b*). Although Paolini *et al*. (2005*a*) found no effect of the intake of sainfoin on the abomasal parasite *H. contortus* in goats; Heckendorn *et al*. (2006, 2007) showed an effect of sainfoin on the same parasite in sheep, when animals were offered sainfoin from 27–28 days post-infection, respectively.

Despite these previous studies on the anti-parasitic activity of sainfoin, the mechanisms of its action are unclear. Both PSM and, in particular, condensed tannins, which are the active compounds in sainfoin (Hoste *et al*. 2005; Paolini *et al*. 2005*b*) may act directly and/or indirectly on the parasites by enhancing the host immune response. Therefore, the purpose of the present study was to further investigate the effect of sainfoin consumption on nematodes and, in particular, to define its mode of action in sheep trickle infected with *T. colubriformis*.

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Table 1. Chemical composition of a pooled sample of the grass and sainfoin hays that were offered to animals that were infected, or not, with *Trychostrongylus colubriformis*

(% phenolics and tannin concentrations are expressed on a dry matter basis.)

	Grass hay	Sainfoin hay
Dry Matter (g/kg fresh matter)	836	886
Crude Protein (g/kg DM)	83	93
ME (MJ/kgDM)	8·8	8·4
FME (MJ/kgDM)	8·6	8·1
DOM (D-value, % DM)	54·9	52·6
Ash (g/kg DM)	70	51
Phosphorus (g/kg DM)	2	1·4
Total Phenolics (Catechin equivalents) (%)	0·8	1·8
Total Tannins (Quebracho equivalents) (%)	<2·0	2·0

ME = Metabolizable energy, FME = Fermentable metabolizable energy estimated by *in vitro* gas production, DOM = Digestive Organic Matter.

MATERIALS AND METHODS

Animals, housing and feeds

Thirty-two weaned 16-week-old Texel × Scottish Greyface lambs previously reared indoors under conditions that excluded exposure to nematodes, were allocated to 1 of 4 groups ($n = 8$) that were balanced for sex (castrated males and females) and initial liveweight (34·5 ± 0·124 kg). Within the animal house, animals were randomly allocated individual pens. From day −14, two of the groups were offered grass hay (diet G) while the remaining 2 groups were offered sainfoin hay (diet S), the chemical composition of each diet is given in Table 1. All feeding was *ad libitum* and animals were also given access to fresh water.

Experimental design

Within each feeding regime, 1 group (infection +) received a trickle infection with 12 000 *Trichostrongylus colubriformis* L3 infective larvae (L_3) per week from day 0 until day 42 that was administered in 3 doses of 4000 larvae per dose on alternate days. The remaining group within each dietary regime was maintained as a non-infected control (infection −). This resulted in a 2 × 2 factorial design with the groups being G+, G−, S+ and S−.

Measurements and sampling procedures

Food composition. Pooled weekly samples of the hays offered and refused were taken and analysed for determination of crude protein (Kjeldahl method), total phenolics (Price and Butler, 1977) and extractable condensed tannin content (Porter *et al*. 1986). Estimation of the energetic feed value and digestibility of the feeds was obtained through *in vitro* gas production using rumen fluid (Menke and Steingass, 1988).

Food intake and liveweight. Fresh hay intake was measured daily with subsamples of feed offered and refused dried to allow estimates of daily dry matter (DM) intake. Lambs were weighed weekly, during the morning of the same day and also on the final day of the experiment prior to slaughter for the estimation of the liveweight gain.

Plasma analysis. Blood samples were collected weekly by jugular venepuncture into heparinized vacutainers (Becton Dickinson, UK). Blood tubes were centrifuged at 2500 ***g*** for 15 min with the plasma removed and stored at −20 °C until analysis. Analysis for plasma total protein, albumin and inorganic phosphate was performed using a microcentrifugal analyser (Monarch 2000, Instrumentation Laboratory, Warrington, Cheshire, UK).

Parasitological measurements. Weekly faecal samples were collected directly from the rectum of all the experimental animals for the determination of the concentration of nematode eggs in the faeces (FEC) by a modified flotation technique (Christie and Jackson, 1982) and were expressed as eggs per g of fresh faeces. Total worm egg output (TEO) was calculated using the measurements of feed intake, digestibility and FEC and an estimate of faecal dry matter using the formula TEO = Total daily faecal DM × FEC/faecal DM %; where: Total daily faecal DM = DM intake × (1-DM digestibility). All animals were slaughtered on day 42. At the time of slaughter, worm recovery from the digesta and the intestinal mucosa of the small intestine following incubation in physiological saline at 37 °C for 4 h was performed on all infected animals. Worm burdens and the ratio of male to female adult worms were counted from a 1% aliquot and multiplied by 100 to give total worm numbers. Adult female fecundity was calculated by dividing the TEO on day 42 by the number of adult female worms present at slaughter and expressed as eggs per female per day).

Histochemical and immunohistochemical analysis. Samples of intestinal tissue from parasitized animals were removed immediately following slaughter and fixed in either PBS containing 4% paraformaldehyde for 6 h at room temperature (Newlands *et al*. 1984) or in zinc salt fixatives at room temperature for 24 h (Gonzales *et al*. 2001). After fixation, tissues were processed and embedded in paraffin;

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5 μm sections were cut, mounted on slides and dried for 12 h at 40 °C. For paraformaldehyde-fixed sections, general histochemical characteristics were assessed following haematoxylin-eosin staining. Mast cells and eosinophils were enumerated following staining with toluidine blue (Enerback, 1966) and carbol-chromotrope (Lendrum, 1944), respectively. Goblet cells in the epithelium and paneth cells in the crypts were detected using Periodic acid-Schiffs (PAS) (Mantle and Allen, 1978). Neutral and acidic mucins were identified through alcian blue-PAS staining (Newlands *et al*. 1990). Stained cells were enumerated using an ×10 eye-piece containing a calibrated graticule and a ×40 objective lens. Zinc salt-fixed tissue sections were employed for immunostaining. Pan T cells were detected with anti-CD3 (AntiCD3, Dako Ltd, Ely, UK) at a dilution of 1 : 100. T-Helper cells were detected using mouse monoclonal anti-ovine CD4 (Basel Institute of Immunology) at a dilution of 1 : 1000. Controls consisted of tissue sections where the primary antibody was omitted. Antibody detection employed the Envision amplification system, using anti-mouse immunoglobulin-horse-radish peroxidase and the substrate DAB, according to the manufacturer's instructions (Dako Ltd, Ely, UK). Stained cells were enumerated using Cell^F (Olympus imaging software, Copyright 1986–2006) that contained a calibrated graticule. All cell counts from both paraformaldehyde- and zinc salt-fixed tissue sections were made systematically in the epithelium and mucosa from the mean of 10 graticule fields, and were expressed as cells/mm^2 of intestinal tissue.

Statistical analysis

Analyses were performed using Genstat statistical software version 7.2 (Lawes Agricultural Trust, 2004) as a 2 × 2 factorial design with diet and infection as the factors, unless otherwise stated. Prior to statistical analysis, faecal egg count (FEC), total worm egg output (TEO), worm burdens were log-transformed (log10 (x + 1)) and intestinal tissue cell concentrations were square-root transformed to remove positive skewness. All transformed data are presented as back-transformed means, unless otherwise stated. Feed intake, liveweight, FEC, TEO and serum analysis underwent sequential comparison of ante-dependence structures for repeated measures before being analysed by Restricted Maximum Likelihood (REML) with time included as a factor. Worm burdens, liveweight gain and intestinal tissue cell concentrations were analysed by using a general Analysis of Variance (ANOVA). For FEC, TEO, worm burden and cell concentrations, analysis was performed on data from infected animals; consequently infection was removed as a factor.

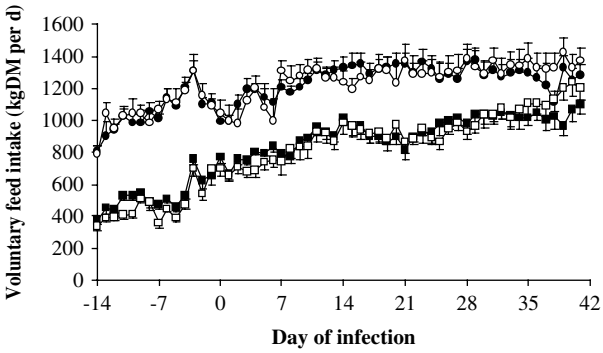

Fig. 1. Means (±S.E.M.) of voluntary dry matter intake (kg/day) of animals that were either offered grass hay and infected with *Trychostrongylus colubriformis* (■), offered grass hay and not infected (□), offered sainfoin hay and similarly infected (●) or offered sainfoin and not infected (○).

RESULTS

Food composition

The two diets were of poor quality and were similar for most of the characteristics measured, with the exception of grass hay containing slightly less crude protein and considerably less total phenolics and total condensed tannins (Table 1).

Food intake

The means for DM intake (DMI) per treatment are shown in Fig. 1. Overall, for feed intake there was a diet × time interaction ($P < 0{\cdot}001$) that reflected a mean feed intake of sainfoin of 1211 g/d that did not differ with time, while intakes of grass-fed animals increased from a mean of less than 500 g/d from day −14 to a mean greater than 1000 g/d from day 29. In addition, there tended to be an infection × diet × time interaction ($P = 0{\cdot}069$) due to infection causing a 14% reduction of feed intake in grass-fed animals from day 39 compared with a 7% reduction observed in sainfoin-fed animals.

Liveweight gains of the animals

Overall, for liveweight there was a diet × time interaction that reflected a divergence in liveweight that increased with time. Liveweight gains during the infection period (from day 0 to day 42) were reduced by infection ($P = 0{\cdot}005$) and were lower in grass-fed than in sainfoin-fed animals ($P = 0{\cdot}002$). However, there was no significant infection × diet interaction with mean liveweight gains during the infection period being 10·6 ± 14·4, 69·1 ± 11·4, 75·2 ± 8·8 and 107·4 ± 22·8 g/day for G+, G−, S+ and S− groups, respectively, ($P = 0{\cdot}38$).

Blood samples

Serum total protein concentration displayed a diet × time interaction ($P = 0{\cdot}05$) that was reflected in

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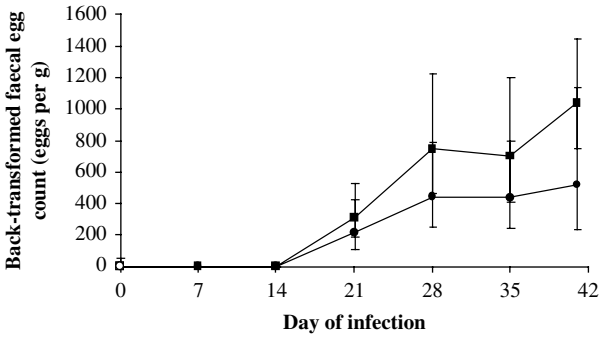

Fig. 2. Mean backtransformed faecal egg counts (FEC; eggs/g) for parasitized animals that were offered either grass hay (■) or sainfoin hay (●). Error bars represent 95% confidence intervals.

similar pre-infection concentrations of 68·3 ± 5·39 and 69·4 ± 5·28 g/l that were maintained in sainfoin-fed animals but decreased in grass-fed animals from day 29, with mean concentrations on day 42 being 67·8 ± 6·73 and 64·1 ± 4·83 g/l for sainfoin- and grass-fed animals, respectively. For serum albumin, there were infection × time ($P = 0·014$) and diet × time ($P = 0·003$) interactions. The former was reflected in similar pre-infection albumin concentrations of 40·5 ± 2·02 and 40·1 ± 1·41 g/l for infected and non-infected animals, respectively, that were decreased at day 42 in infected but not in non-infected animals, namely, 35·3 ± 2·80 and 37·8 ± 2·32 g/l for infected and non-infected, respectively. The latter interaction was reflected in similar pre-infection concentrations of 40·4 ± 1·86 and 40·2 ± 1·65 g/l for grass- and sainfoin-fed animals that were decreased in grass-fed animals from day 22, resulting in mean concentrations at day 42 of 35·8 ± 2·76 and 37·4 ± 2·74 g/l for grass- and sainfoin-fed animals, respectively.

Overall, serum phosphate concentrations were greater in sainfoin-fed animals, namely, 4·60 ± 1·60 and 5·16 ± 1·53 mg/dl for grass- and sainfoin-fed animals, respectively ($P < 0·001$). In addition, there was an infection × time interaction ($P < 0·001$) that reflected similar concentrations at day −14 of 4·14 ± 1·13 and 4·48 ± 0·89 mg/dl in infected and non-infected animals, respectively, that were decreased by a mean of 30% in infected animals from day 15, with concentrations at day 42 being 3·34 ± 0·94 and 5·59 ± 1·28 mg/dl for infected and non-infected animals, respectively.

Parasitological measurements

Back-transformed faecal nematode egg counts (FEC) are given in Fig. 2. Overall, there was an effect of time and diet ($P < 0·001$ for both) with an increase in the concentration of eggs with time and greater concentrations observed in grass-compared with sainfoin-fed animals. Due to the differences in feed intake between sainfoin- and grass-fed animals, total daily nematode egg output (TEO) was estimated. For TEO, egg output increased with time ($P < 0·001$) from 526 017 on day 21 to $1·1 \times 10^6$ on day 42 but was not affected by diet ($P = 0·46$).

Table 2. Arithmetic mean ± S.E. for numbers of worms and adult female fecundity (eggs/female/day) of *Trychostrongylus colubriformis* worms that were retrieved from animals after 42 days of infection and offered either grass hay or sainfoin hay

(Lower (25%) and upper (75%) quartile threshold values are given in parenthesis. Statistical comparisons for worm numbers were performed on log10 transformed data. No significant differences for any parameter were detected.)

	Grass hay	Sainfoin hay	s.e.m
Juvenile	6525 ± 2302 (4550–8600)	5625 ± 1653 (4750–6225)	
Adult female	19 513 ± 5238 (14 950–24 150)	21 263 ± 6959 (16 400–23 075)	
Adult male	16 775 ± 6310 (10 325–22 000)	19 063 ± 6556 (13 225–22 525)	
Total	42 813 ± 12 084 (33 625–54 350)	45 950 ± 13 069 (38 175–50 800)	
Adult female fecundity (eggs per female per d)	73·8 ± 28·4 (55·3–100·8)	57·5 ± 28·5 (32·3–83·0)	10·08

Worm burdens recovered from the small intestine are shown in Table 2. Overall, there was no effect of diet on the number of juvenile worms ($P = 0·43$), adult males ($P = 0·46$), adult female ($P = 0·57$), total adult burdens ($P = 0·48$), total worm burdens ($P = 0·58$) or female adult fecundity ($P = 0·27$).

Intestinal tissue cell counts

The numbers of immunological cells per mm² of intestinal tissue in parasitized animals at slaughter are given in Table 3. Compared to their grass-fed counterparts, intestinal tissues of sainfoin-fed animals had greater concentrations of both Pan T cells ($P = 0·01$), mast cells ($P = 0·04$) and eosinophils ($P < 0·001$). In addition, sainfoin-fed animals had similar concentration of T helper cells ($P = 0·25$), Paneth cells ($P = 0·15$) and goblet cells in both the epithelium ($P = 0·26$) and crypt ($P = 0·22$). Of the goblet cells, there was no difference between diets in the concentration of cells containing neutral mucin ($P = 0·54$). However, there was a tendency for a greater concentration of goblet cells containing acidic mucins (alcian blue positive) in grass-fed animals ($P = 0·13$), with these alcian blue-positive cells comprising 39% and 25% of the total number of goblet cells for grass- and sainfoin-fed animals, respectively.

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Table 3. Arithmetic mean ± S.E concentrations (cells/mm^2) of T cell subsets, mast cells, eosinophils, goblet cells, Paneth cells and mucin-producing cells in intestinal tissues retrieved from lambs after 42 days of infection with *T. colubriformis* while offered either grass or sainfoin hay

(Lower (25%) and upper (75%) quartile thresholds are given in parenthesis. Statistical comparisons were made on square-root transformed data.)

					Goblet cells			Mucin-producing cells		
	Pan T cells	T helper cells	Mast cells	Eosinophils	Epithelium	Crypts	Paneth cells	Neutral	Mixture	Acid
Grass Hay	579 ± 155 (471–667·1)	111 ± 80·4 (18–183)	24·7 ± 15·8 (8·6–33·7)	11·4 ± 7·9 (3·8–17·9)	37·9 ± 32·4 (9·8–61·6)	104 ± 58·3 (70–143)	87·7 ± 48·4 (52·7–132)	14·6 ± 9·8 (7·9–19·4)	8·2 ± 5·2 (4·8–10·1)	13·5 ± 7·9 (7·0–22·4)
Sainfoin Hay	741 ± 79·7 (675–822)	153 ± 70·9 (104–167)	44·4 ± 20·1 (26·4–58·3)	41 ± 16·1 (33·6–53·5)	54·6 ± 13·2 (43·5–63·6)	136 ± 18·2 (121–149)	122 ± 21·3 (104–144)	16·7 ± 7·3 (11–21·5)	9·1 ± 3·8 (6·0–11·9)	8·2 ± 4·4 (4·6–12·3)
	$P = 0·01$	NS	$P = 0·04$	$P < 0·001$	NS	NS	NS	NS	NS	NS

DISCUSSION

In the present study, attempts were made to address the anthelmintic properties of sainfoin hay, and to investigate possible modes of action. Although feeding sainfoin did not have significant effects on worm burdens or total egg output, an intriguing finding was that sainfoin enhanced some local cellular responses associated with GI nematode immunity (Miller, 1984). This result suggests that components within sainfoin are able to enhance the normal local pathological and/or immune responses which occur during infection, either directly, or through improved nutrition.

In contrast to the present results, studies in adult goats using 5% quebracho (Paolini *et al*. 2003*a*) or sainfoin hay containing 2·7% CT (Paolini *et al*. 2005*b*) showed a clear effect of sainfoin hay consumption on *T. colubriformis* egg excretion and worm burden, although no such effects were observed for *H. contortus* infections when sainfoin hay containing 3·2% was offered (Paolini *et al*. 2005*a*). Furthermore, using naïve lambs infected with *T. colubriformis*, Athanasiadou *et al*. (2005) found that animals offered lucerne hay had a significantly higher egg output than those animals receiving sainfoin hay. The studies of Heckendorn *et al*. (2006, 2007) were the first to find dramatic results of sainfoin on *H. contortus* of sheep and some minor effects on *Cooperia curticei*. In the first study, Heckendorn *et al*. (2006) reported a reduction in FECDM (faecal egg count on a dry matter basis) by 58% in the group offered sainfoin hay that contained 6·1% CT and a 48% reduction in the FECDM in the group offered sainfoin silage containing 4·2% CT when compared to the FECDM of the group offered control diets containing 0·1% CT. In the second study, Heckendorn *et al*. (2007) found a significant reduction in the total daily faecal egg output for *H. contortus* of 89% for animals fed with chicory containing 0·3% CT and 63% for those fed with birdsfoot trefoil and sainfoin containing 1·5% and 2·6% CT, respectively, indicating that the effect found with chicory could be due to a PSM other than condensed tannins. However, in the present study, worm burdens and fecundity were not affected by the feeding treatment of the animals in the time frame (6 weeks) investigated. It appears from the literature that differences related to the source (plant species), CT content, changes in CT content within species, species of the parasite and of the host, and time of exposure to both parasites and PSM are all factors that may influence the anthelmintic effect. Perhaps the latter aspect is of particular relevance in the present study, where animals were challenged with parasites for a relatively short (6 weeks) period. Previous studies have shown that *Trichostrongylus* populations are not subject to obvious regulation, including falls in egg counts or worm burdens, until after 6 weeks of

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exposure to infection (Seaton *et al*. 1989; Bown *et al*. 1991; Coop *et al*. 1995). While sainfoin induced increases in local inflammatory and immune cell populations, these were clearly not sufficient to result in a reduction in parasite worm burdens or egg production. However, since worm immunity is a dynamic and complex process, it is possible that a longer exposure to the parasites and sainfoin may have resulted in a more rapid, or of a greater magnitude, immune-mediated response.

There is much evidence for the important role of mast cells and epithelial globule leukocytes in the local immune response of rodents and sheep to parasites (Miller, 1984; Huntley, 1992; Balic *et al*. 2000). However, the ability of PSM to influence these inflammatory responses to GI parasites has not previously received much attention. In previous work by Paolini *et al*. (2003*a*), drenching goats infected with *H. contortus* with quebracho, a high tannin-containing extract of the tropical tree *Schinopsis quebracho*, did not modify the eosinophils, mast cells or globule leukocytes in the abomasal tissue. However, administration of extract was relatively short term. In a different study in growing lambs, Tzamaloukas *et al*. (2006) showed that animals grazing sulla or chicory had an enhanced immunity against *T. circumcincta*, with elevated counts for globule leukocytes and mucosal mast cells in abomasal tissues, but no differences for eosinophils. However, the results found in the previous two studies are difficult to compare because of the differences of host species, parasite species, source of PSM and experimental design used.

Both eosinophils and mucin-producing goblet cells have also been implicated in the protective effector immune responses against GI nematode parasites. Eosinophils may damage or kill gastrointestinal nematodes larvae *in vivo* (Balic *et al*. 2006), and goblet cell hyperplasia is a characteristic feature of infection (Ishikawa *et al*. 1997). In addition to the increase in goblet cells, studies in sheep have shown that the quality of mucins has also been shown to change, from an essentially neutral to acidic composition during the acquisition of immunity (Newlands *et al*. 1990). These changes may enhance the exclusion of larvae, by trapping and preventing them from entering the crypt regions (Miller *et al*. 1981), possibly by increasing the viscosity or physical nature of the mucin, or enhanced binding of specific anti-worm proteins such as IgA antibodies or intelectin (Pemberton *et al*. 2004). However, there appeared to be no quantitative changes in these cells between sainfoin- and grass-fed animals in the time frame investigated. Previous work has shown that goblet cell hyperplasia is controlled by T cells (Khan and Collins, 2004), and a significant increase in Pan T cells was demonstrated in the lamina propria in this study. While T helper cells are known to be principally involved (Khan and Collins, 2004), the identity and proportions of individual T cell subpopulations were not determined in this work.

A range of nutritional components are known to influence the cells associated with the acquisition and expression of immunity including protein and energy (Coop and Kyriazakis, 2001). Both hays were considered in terms of protein and energy as being relatively similar but poor nutrient sources (see Table 1). However, the greater feed consumption of sainfoin makes the possible direct nutritional effect and that of a direct action of PSM's difficult to separate. Post-ruminal infusions of casein supplying 50 g of metabolizable protein (MP) per day, in addition to a basal diet of approximately 50 g MP per day, have been shown to enhance the rate of acquisition of immunity (Bown *et al*. 1991). Unfortunately Bown *et al*. (1991) did not monitor tissue cell counts; however, these authors did not observe any difference in worm burden after 6 weeks, but a reduced worm burden after 12 weeks of infection in casein-supplemented animals of a comparable age and infected with the same nematode species as used in the current study. During the course of the infection, sainfoin-fed animals consumed a mean of 117 g crude protein (CP) per day in comparison to the 77 g CP consumed by their grass-fed counterparts. Therefore, it may be possible that the enhanced immunological activity observed in the tissue retrieved from sainfoin-fed animals was a reflection of the greater nutrient supply allowing an earlier acquisition of inflammatory responses that are associated with immunity (Miller, 1984). Comparable to the aforementioned results of Bown *et al*. (1991), the expression of such immune mechanisms, in terms of reduced parasite burden and/or parasite fecundity, would not be expected given the short time-frame of infection.

Despite this difference in total nutrient intake, there is a plethora of evidence to support the immuno-stimulatory properties of PSMs. In particular, lectins are PSMs that have known immuno-stimulatory properties and have been widely employed to non-specifically activate T cells inducing T cell proliferation (Kay, 1991). Although lectins have been isolated from sainfoin (Young *et al*. 1982) the properties of this protein have not yet been studied. Clearly, further fractionation studies will be required to attempt to identify which components in sainfoin are responsible for the local cellular responses observed – a challenging task given the huge range of proteins and bioactive molecules that will be present.

In summary, these results support the notion that feeding sainfoin hay may act to enhance the inflammatory and immune cell response to GI nematodes. From the current study it is unclear if this is due to enhanced nutrition supplied by the sainfoin or if it is due to the direct immuno-stimulatory properties of PSMs. However, at this relatively early stage in the

development of acquired immunity there did not appear to be any significant reductions in worm burden or total egg output. Nevertheless, the present study adds to the increasing evidence that dietary factors are able to influence the host's local immune reaction to intestinal parasites, although further studies would be required to define the active components in sainfoin and to elucidate their mode of action.

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