Fructosamine concentration and resistance to natural, predominantly *Teladorsagia circumcincta* infection

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SUMMARY

Fructosamine concentrations reflect protein status and because infection with *Teladorsagia circumcincta* can induce a relative protein deficiency, we examined the usefulness of fructosamine concentrations as markers of the intensity of infection in naturally infected lambs. Fructosamine concentration was a heritable trait and variation in fructosamine concerntrations was associated with differences in body weight, and a variety of parasitological variables; animals with increased fructosamine concentrations grew more quickly, had increased faecal egg counts in one of the three study years, had decreased pepsinogen concentrations and decreased IgA activity against 4th-stage larvae of *T. circumcincta*. Fructosamine concentrations were also associated with variation in the subsequent acquisition of nematodes and in the length of adult female *T. circumcincta*; lambs with increased fructosamine concentrations had fewer nematodes but the mean length of adult female *T. circumcincta* was longer. Therefore fructosamine concentrations are potentially useful indicators of the severity of nematode infection and may predict magnitude of subsequent infection.

Key words: sheep, Nematoda, *Teladorsagia circumcincta*, fructosamine, marker, indicator trait.

INTRODUCTION

Nearly all sheep grazing in temperate areas are infected with the abomasal nematode *Teladorsagia* (*Ostertagia*) *circumcincta*. Variation in resistance to this parasite appears to be an important source of variation in growth rate among lambs (Bishop *et al*. 1996; Bouix *et al*. 1998). However, resistance to infection can be difficult to determine on commercial farms. A common procedure is to compare differences in the magnitude of infection among lambs with similar histories of exposure. Faecal egg counts are commonly used to assess infection intensity but the relationship between egg counts and the intensity of infection is complex (Stear *et al*. 1997; Bishop & Stear, 2000). Additional markers to assess infection status are needed.

One possible marker is fructosamine concentration. Infection with *T. circumcincta* can produce a relative protein deficiency; the clinical signs can be alleviated by diets rich in protein (Coop & Holmes, 1996; Coop & Kyriazakis, 1999). Fructosamines are stable covalent ketoamines formed by the nonenzymatic reaction of glucose with amino groups on proteins (Bernstein, 1987). Their concentration reflects average glucose and protein concentrations as well as rates of protein turnover. Protein turnover increases with nematode infection, and Heath & Connan (1991) have shown that fructosamine concentrations decrease following deliberate gastrointestinal infection. However, the value of fructosamine concentration has yet to be determined in natural infections. The aim of our study was to assess whether fructosamine concentrations reflect variation among sheep in the intensity of natural, predominantly *T. circumcincta* infection. The measurements for comparison with fructosamine concentration were faecal egg count, number of nematodes present in the gatrointestinal tract, mean length of adult female *T. circumcincta* (Stear & Bishop, 1999), plasma IgA activity against 4th-stage larvae of *T. circumcincta* (Stear, Strain & Bishop, 1999*b*), plasma pepsinogen concentration (Stear *et al*. 1999*a*) and bodyweight (Bishop *et al*. 1996).

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MATERIALS AND METHODS

Animals

The lambs came from a commercial, upland farm in Southwest Strathclyde. This farm has been described previously (Stear *et al*. 1995). In brief, all sheep were straightbred Scottish Blackface and all husbandry procedures followed standard commercial practice. Each year a cohort of 200 predominantly twin-born lambs were studied but only 109,

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Table 1. Arithmetic mean ± standard deviation of body weight, faecal egg count, plasma pepsinogen concentration and IgA activity against 4th-stage larvae among lambs in September

(Pepsinogen concentrations and IgA activities are expressed as percentages of an internal standard.)

Year	Body weight (kg)	Faecal egg count (epg)	Pepsinogen concentration	IgA activity
1992	25 ± 3	268 ± 456	50 ± 44	0·20 ± 0·14
1993	31 ± 4	190 ± 239	31 ± 20	0·24 ± 0·16
1994	31 ± 4	95 ± 138	21 ± 17	0·18 ± 0·17

100 and 153 lambs were slaughtered in 1992, 1993 and 1994 respectively. The lambs in this study were born in the last 2 weeks of April and the first week of May. Within 1 week of lambing, the ewes and lambs were moved onto 1 of 3 fields close to the farmhouse. At 3 or 4 months of age, the lambs were separated from their mothers and moved onto the largest of the 3 fields. All lambs were given a broad spectrum anthelmintic (albendazole sulphoxide) at the dose rate recommended by the manufacturer every 28 days from 4 to 20 weeks of age. Blood and faecal samples were taken every 28 days at the same time as anthelmintic treatment. All animals were also weighed on the same day as anthelmintic treatment. No additional blood samples were collected although faeces from 6 sheep with high faecal egg counts were collected once every year after anthelmintic treatment to test for anthelmintic resistance. The mean body weights in September 1992, 1993 and 1994 are given in Table 1. Half of the lambs slaughtered each year were killed 6 weeks after the final anthelmintic treatment; the remainder were killed after 7 weeks. All lambs were slaughtered at the local abattoir.

Fructosamine concentrations

The fructosamine concentration in plasma samples was measured using a Cobas Mira discrete biochemical analyser with a commercial kit (Unimate Fructosamine) and calibrated with a specific glycated polylysine calibrant, all of which were obtained from the same supplier (Roche Diagnostics Ltd). Fructosamine concentrations were measured in July, August and September 1992 and in September 1993 and September 1994.

Parasitological assessments

Standard parasitological procedures were used for faecal egg counts and to enumerate all nematodes in the abomasum and small intestine (Armour, Jarrett & Jennings, 1966). Twenty-five adult female worms, chosen at random from each infected lamb, were measured by image analysis (PC-Image, Foster Findlay Associates Ltd) to determine mean worm length. As female worms are slightly longer than male worms and sheep with longer females also have longer males (unpublished observations), only female worms were measured. Faecal egg counts excluded *Nematodirus* spp. The large intestine was not examined as no large intestinal parasites appeared to be present on these fields, possibly because of the frequency of anthelmintic treatment (Stear *et al*. 1998). A faecal egg count reduction test was carried out on at least 6 animals with high egg counts in June every year of the experiment. The reduction in faecal egg count was always greater than 95 % and there was no indication of anthelmintic resistance during this study. The mean faecal egg count varied among months and years and there was no discernible pattern to the mean egg counts. The means for September 1992, 1993 and 1994 are given in Table 1. The mean egg counts at slaughter are presented in Table 2. Five taxa were responsible for the vast majority of adult and larval nematodes recovered at slaughter (Stear *et al*. 1998): *T. circumcincta*, *Trichostrongylus axei*, *Trichostrongylus vitrinus*, *Cooperia* spp. and *Nematodirus* spp. The arithmetic mean numbers for the different taxa in 1992, 1993 and 1994 are given in Table 2. Faecal egg counts and nematode numbers were log-transformed (log 10 $x+1$) prior to analysis.

Pepsinogen concentrations

The procedures used were as described by Stear *et al*. (1999*a*). In brief, the method of Paynter (1992) was adapted for small quantities. The internal standard was created by pooling plasma from infected sheep. Its pepsinogen concentration was 3·4 ± 0·1 (mean ± standard deviation in international units, $n = 11$) by the method of Edwards, Jepson & Wood (1960) and 10·8 ± 3·0 international units ($n = 12$) by the modification of Paynter's (1992) method described here. Scott, Stear & McKellar (1995) reported that Paynter's method gave values about 5 times higher than the method of Edwards *et al*. (1960). To simplify comparisons we have presented the pepsinogen concentrations as a percentage of the internal standard. The mean pepsinogen values are given in Table 1. Pepsinogen concentrations were log-transformed (log 10 $x+1$) prior to analysis.

IgA responses

The activity of plasma IgA against a somatic extract of 4th-stage larvae from *T. circumcincta* was measured by indirect ELISA. Fourth-stage larvae were

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Table 2. Arithmetic mean faecal egg count and mean number of nematodes recovered at necropsy in October and November – 6 or 7 weeks after anthelmintic treatment

Year	Faecal egg count (epg)	*T. circumcinta*	*T. axei*	*Cooperia* spp.	*T. vitrinus*	*Nematodirus* spp.
1992	79	12936	0	84	256	231
1993	320	3406	68	388	116	456
1994	479	2301	1	562	257	120

harvested 4 days after infecting helminth-naïve lambs with 150000 infective larvae. The abomasum was washed with tap water and cut into strips. These strips were suspended in Baermann funnels containing PBS (pH 7·4) at 37 °C. Larvae were collected and allowed to migrate through surgical gauze into PBS. The recovered larvae were washed 5 times in PBS, once in PBS containing 100 i.u. pencillin/ml, 0·1 mg streptomysin/ml, 2·5 μg amphotericin B/ml, and 0·05 mg gentamicin/ml and once in Tris-inhibitor solution (10 mM Tris containing 1 mM EDTA (disodium ethylene diamine tetraacetic acid), 1 mM EGTA (ethylene glycol *bis* (2-amino ethyl ether) –N,N,N′N′-tetraacetic acid), 1mM NEM (N-ethylmaleimide), 0·1 μM pepstatin, 1 mM PMSF (phenyl methyl sulphonyl fluoride) and 0·1 mM TPCK (N-tosylamide-L-phenylalanine chloromethyl ketone)). After centrifugation, the pellet was resuspended in 1% (v/v) sodium deoxycholate in Tris-inhibitor solution and stored at −20 °C. After thawing, the sample was homogenized on ice with a hand-held electric homogenizer (Janke & Kunkel, IKA Labortechnik). The supernatant was filtered through a 0·2 μm filter and aliquots stored at −80 °C. The protein concentration was estimated with Bicinchoninic acid (Pierce) and adjusted to 5 μg/ml in 0·06 M bicarbonate buffer (pH 9·6) before use.

The wells on flat-bottomed microtitre plates (Nunc) were coated with 100 μl of parasite solution and left overnight at 4 °C. The plate was washed 5 times in PBS–Tween (0·1% (v/v) Tween 20 in PBS), incubated for 2 h at 37 °C with 200 μl of blocking buffer (4% skimmed milk powder in PBS–Tween), then again washed 5 times in PBS–Tween. A vol. of 100 μl of plasma sample, diluted 1:10 in blocking buffer, was added to each of 3 wells and incubated at 37 °C for 30 min. After another 5 washes in PBS–Tween, 100 μl of a rat monoclonal anti-sheep IgA, at a dilution of 1:50 in blocking buffer, was added and incubated for 30 min at 37 °C. After a further 5 washes in PBS–Tween, 100 µl of goat anti-rat IgG conjugated to alkaline phosphatase (Sigma) at a dilution of 1:1000 in blocking buffer was added and incubated for 30 min at 37 °C. After a final 5 washes in PBS–Tween, 100 μl of 5-bromo-4-chloro-3-indoylphosphate (BCIP) (Kirkegaard and Perry Laboratories) were added and incubated for a further 30 min at 37 °C. The reaction was then read on a microplate reader at 635 nm. The mean of 3 replicates from a pooled sample of helminth-naïve lambs was subtracted from the sample mean and this adjusted mean was divided by the mean of 3 replicates from a pool of high-responder lambs after subtracting the mean of the helminth naïve lambs (Sinski *et al*. 1995). Three replicates from the naïve and high responder pools were included on each plate. The pool of high responder lambs was created by combining equal quantities of plasma from 6 lambs that gave strong IgA responses following natural infection. The value for each lamb was therefore expressed as a proportion of a positive control. Mean IgA activity in September is presented in Table 1. IgA activities were log-transformed (log 10 $x+1$) prior to analysis.

Statistical analyses

The mean values and standard deviations were obtained from the MEAN procedure on the SAS suite of computer programs (SAS Institute, Cary, N. Carolina). The Shapiro-Wilk procedure on the UNIVARIATE procedure (SAS) was used to test for deviations from a normal distribution. The GENERAL LINEAR MODELS procedure was used for variance analyses. Covariance models can produce potentially confusing results when the model includes a covariate such as fructosamine concentration as a main effect and as an interaction with year. The solution produced by SAS sets the interaction term with the highest label (here fructosamine*1994) to zero and compares the other 2 interaction terms against the zero term. One consequence of this is that the solution for the main effect is quite different from models without the interaction term. In order to facilitate comparisons between models we have used the estimate statement to determine the value of the main effect and have estimated the interaction of fructosamine with year from the solution in a model that lacked the main effect of fructosamine. The ASREML package (ftp.res.bbsrc.ac.uk/pub/aar) was used to estimate the heritability and standard error of fructosamine concentrations with an animal model that included the known pedigree relationships and the effects of year and sex.

A series of variance analyses was used to examine the associations between fructosamine concentrations and body weights, faecal egg counts, pep-

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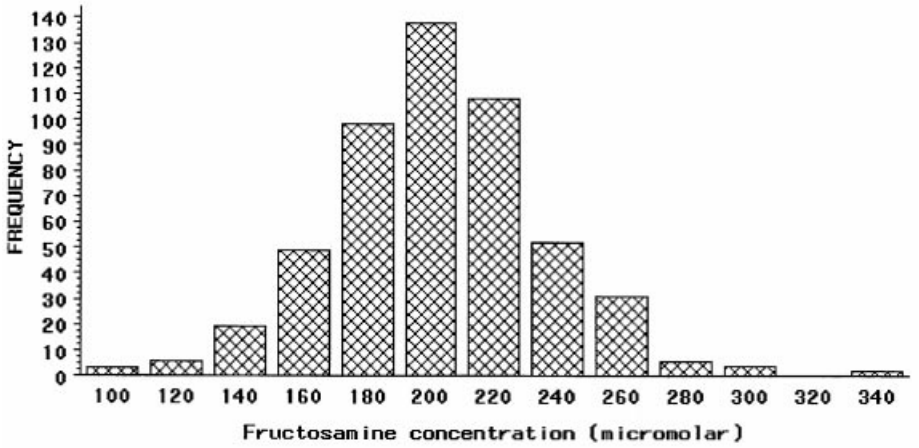

Fig. 1. The distribution of fructosamine concentrations in plasma among naturally infected sheep in September. Fructosamine concentrations are expressed in μmol/l.

Table 3. Covariance analysis of body weight among lambs in September

(The R-square was 0·33 and data from 511 lambs were analysed. The initial analysis indicated that the square of the fructosamine concentration was not significant at the 5% level ($P = 0{\cdot}500$). This term was discarded from the final model.)

Variable	Probability		Effect
Intercept	0·001		29·8 ± 0·39
Year	0·001	1992	−4·70 ± 0·53
		1993	0·21 ± 0·44
		1994	0
Sex	0·002	Male	1·07 ± 0·36
		Female	0
Fructosamine concentration	0·001		0·030 ± 0·01
Fructosamine concentration*year	0·046	1992	0·015 ± 0·01
		1993	0·018 ± 0·01
		1994	0·057 ± 0·02

sinogen concentrations, IgA activity, total numbers of nematodes and the length of adult female *T. circumcincta*. These assessments used fructosamine concentrations measured on the September samples. Body weights, plasma samples and faecal samples were collected on the same day. The number of nematodes and the mean length of adult female *T. circumcincta* were estimated from samples taken at necropsy 6 or 7 weeks later. All traits were initially analysed with models that included year and sex as fixed effects and fructosamine concentrations, the interaction between year and fructosamine concentration and the square of the fructosamine concentration as covariates. The squared term was used to test for non-linear associations of fructosamine concentration on weight gain or parasitic infection. The mean was subtracted from the fructosamine concentration before estimating the regression coefficients.

RESULTS

Fructosamine concentrations were measured in plasma samples taken from the same lambs in July, August and September 1992. The mean values and their standard errors were 167 ± 19 μmol/l (n = 197), 185 ± 21 μmol/l (n = 190) and 173 ± 30 μmol/l (n = 144) in July, August and September 1992. Additional lambs were sampled in September 1993 and September 1994 with values of 225 ± 31 μmol/l (n = 194) and 202 ± 19 μmol/l (n = 181) respectively. Fig. 1 shows the distribution of fructosamine concentrations pooled across years. The distribution was unimodal, symmetrical and not significantly different from the normal distribution ($P = 0{\cdot}47$). In 1992, the correlations among samples taken in July and August were 0·37 ($P < 0{\cdot}0001$), July and September, 0·29 ($P < 0{\cdot}01$) and August and September 0·20 ($P < 0{\cdot}05$).

The heritability of fructosamine concentrations was estimated with an animal model that included the effects of year ($P < 0{\cdot}001$) and sex ($P < 0{\cdot}01$). The heritability estimate and standard error were 0·34 ± 0·14 ($P < 0{\cdot}05$).

Animals with relatively high fructosamine concentrations were heavier than animals with relatively low fructosamine concentrations (Table 3). For this analysis the samples collected in September were used and blood samples were collected at the same time as the animals were weighed. As expected males were heavier than females. The association of fructosamine concentration and bodyweight was always positive but the strength of the relationship varied among years, being strongest in 1994 and weakest in 1992. The association between log-transformed faecal egg counts and fructosamine concentrations was also assessed by covariance analysis in samples taken on the same day in September; the results from the 3 years were analysed together (Table 4). The association of fructosamine with faecal nematode egg count varied

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Table 4. Covariance analysis of faecal egg count

(The initial analysis indicated that the association with the square of the fructosamine concentrations ($0{\cdot}10 < P < 0{\cdot}20$) and with the main effect of fructosamine ($0{\cdot}10 < P < 0{\cdot}20$) were not significant. The remaining terms together accounted for 12% of the total variation ($n = 487$).)

Variable	Probability		Effect
Intercept	0·001		0·97 ± 0·10
Year	0·001	1992	0·43 ± 0·14
		1993	0·59 ± 0·12
		1994	0
Sex	0·008	Male	0·26 ± 0·10
		Female	0
Fructosamine concentration*year	0·018	1992	−0·0043 ± 0·0028
		1993	0·0054 ± 0·0023
		1994	0.0064 ± 0·0041

Table 5. Covariance analysis of pepsinogen concentration

(The initial analysis with the full model indicated that the association between the square of the fructosamine concentration and pepsinogen concentration was not significant ($0{\cdot}40 < P < 0{\cdot}50$), neither was the year by fructosamine interaction ($0{\cdot}30 < P < 0{\cdot}40$) nor the effect of sex ($0{\cdot}80 < P < 0{\cdot}90$). The remaining terms accounted for 11% of the total variation in pepsinogen concentration ($n = 505$).)

Variable	Probability		Effect
Intercept	0·0001		1·13 ± 0·05
Year	0·0001	1992	0·32 ± 0·06
		1993	0·29 ± 0·05
		1994	0
Fructosamine concentration	0·0021		−0·0024 ± 0·0008

Table 6. Covariance analysis of plasma IgA activity against 4th-stage larvae of *Teladorsagia circumcincta*

(The initial analysis with the full model indicated that the association between the square of the fructosamine concentration and IgA activity was not significant ($0{\cdot}40 < P < 0{\cdot}50$), nor was the influence of sex ($0{\cdot}20 < P < 0{\cdot}30$). The effects of year, fructosamine concentration and the interaction between year and fructosamine concentration accounted for 7% of the total variation in IgA activity ($n = 508$).)

Variable	Probability		Effect
Intercept	0·0001		0·25 ± 0·01
Year	0·0001	1992	0·04 ± 0·02
		1993	0·09 ± 0·02
		1994	0
Fructosamine concentration	0·0012		−0·00090 ± 0·00029
Fructosamine concentration*year	0·0187	1992	−0·00010 ± 0·00048
		1993	−0·00046 ± 0·00037
		1994	−0·00215 ± 0·00065

among years and was only significant in 1993 when animals with high fructosamine concentrations had high faecal egg counts. The relationship between log-transformed pepsinogen and fructosamine concentrations was assessed on samples taken on the same day in September after pooling across the 3 years of the study (Table 5). Decreased fructosamine concentrations were associated with increased pepsinogen concentrations in plasma and there was no evidence for any variation in this relationship in different years.

The relationship between log-transformed plasma IgA activity against 4th-stage larvae and fructosamine concentrations was also assessed on samples taken on the same day in September after pooling across the 3 years of the study (Table 6). The results indicate that the association of fructosamine with IgA activity was negative but that the strength of the association varied among years.

The log-transformed number of nematodes (adults and larvae of all species) were estimated 6 and 7 weeks after samples were taken for estimation of

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Table 7. Covariance analysis of nematode number

(The 5 variables accounted for 33% of the total variation in the log-transformed number of nematodes acquired after anthelmintic treatment ($n = 313$). Excluding the 2 non-significant terms (fructosamine concentration*year and the square of fructosamine concentration) reduced the r-square value to 31%.)

Variable	Probability		Effect
Intercept	0·0001		3·22 ± 0·06
Year	0·0001	1992	0·55 ± 0·07
		1993	0·11 ± 0·06
		1994	0
Sex	0·0274	Male	0·15 ± 0·07
		Female	0
Fructosamine concentration	0·0320		−0·0019 ± 0·0009
Square of fructosamine concentration	0·0659		0·00003 ± 0·00002
Fructosamine concentration*year	0·0839	1992	0·0009 ± 0·0017
		1993	−0·0019 ± 0·0017
		1994	−0·0046 ± 0·0018

Table 8. Covariance analysis of mean length of adult female *Teladorsagia circumcincta*

(The initial analysis demonstrated that sex ($0·30 < P < 0·40$) and the quadratic term were not significant ($0·50 < P < 0·60$). The remaining variables accounted for 11% of the variation in adult worm length ($n = 315$).)

Variable	Probability		Effect
Intercept	0·0001		0·88 ± 0·01
Year	0·0001	1992	−0·07 ± 0·02
		1993	−0·08 ± 0·02
		1994	0
Fructosamine concentration	0·0183		0·0006 ± 0·0002
Fructosamine concentration*year		1992	−0·0003 ± 0·0004
		1993	0·0004 ± 0·0003
		1994	0·0016 ± 0·0005

fructosamine concentration. As all animals were given an effective anthelmintic at the time of sample collection, this analysis examined the value of fructosamine concentrations in predicting the magnitude of future infection. The negative association demonstrates that animals with low fructosamine concentrations acquired more nematodes (Table 7). This effect was strongest in 1994 and not significantly different from zero in 1992.

The mean length of adult female *T. circumcincta* in different sheep was also estimated in samples obtained at necropsy 6 and 7 weeks after anthelmintic treatment and collection of plasma samples for estimation of fructosamine samples. The overall association was positive, animals with low fructosamine concentrations had shorter worms. As with worm number, the association was strongest in 1994 and not significantly different from zero in 1992 (Table 8).

DISCUSSION

This study has shown that fructosamine concentrations were normally distributed among grazing lambs. There were moderate correlations between samples taken at monthly intervals. Variation in fructosamine concentrations was moderately heritable. When samples were taken at the same time, decreased fructosamine concentrations were associated with decreased bodyweight, decreased faecal egg counts in some but not all years, increased pepsinogen responses and increased parasite-specific IgA responses. In addition, there were significant differences among years in the influence of fructosamine concentration on body weight, egg counts and IgA activity. Lambs with low fructosamine concentrations subsequently acquired more nematodes of all species and had shorter adult female *T. circumcincta*. Therefore, fructosamine concentrations were associated with current levels of nematode infection and also appeared to predict future levels of infection.

The number of adult *T. circumcincta* influences worm length and an increase in the number that establish would lead to shorter worms (Stear *et al.* 1997). Parasite fecundity varies with worm length (Stear & Bishop, 1999). Consequently, faecal egg counts will be influenced by both the number of *T. circumcincta* and their mean length (Stear *et al.* 1997). As the number of *T. circumcincta* increases from zero, egg output rises then falls as the density-dependent decrease in fecundity overcompensates

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for the increase in worm number (Bishop & Stear, 2000). Convex relationships between egg production and parasite number have also been reported for other parasites including schistosomes in cattle (De Bont *et al*. 1991), hookworms in ring-necked pheasants (Tompkins & Hudson, 1999) and nematodes in reindeer (Irvine *et al*. 2000). One feature of the interaction between mean fecundity and the density of *T. circumcincta* in sheep is that egg output is relatively unresponsive to changes in nematode number at moderate levels of infection (Bishop & Stear, 2000). This unresponsiveness could explain why associations between fructosamine concentrations and faecal egg output were not consistently observed in this study.

An increase in the number of *T. circumcincta* ingested could lead to an increase in the pepsinogen response and in the IgA response, as antibody responses are known to be dose dependent. However, the reduced size of *T. circumcincta* at higher parasite densities would reduce the influence of worm number on the dose of parasite antigen. Increased levels of IgA as well as increased numbers of adult worms would decrease worm length and fecundity (Stear *et al*. 1999*b*).

There are perhaps 3 possible explanations for the association between decreased fructosamine concentrations and increased numbers of nematodes at subsequent necropsy: greater appetite, stronger immune response or modified grazing behaviour. However, the association is unlikely to be simply a consequence of increased appetite. For example, in 1994 animals in the top 10 percentile of fructosamine concentrations had an average of 1550 *T. circumcincta* compared to a mean of 3056 among lambs in the bottom 10 percentile of fructosamine concentrations. Lambs with the lowest fructosamine concentrations are unlikely to have eaten twice as much as lambs with the highest fructosamine concentrations. The ability to control the number of *T. circumcincta* that establish appears to be largely or wholly acquired by sheep after the first grazing season (Stear *et al*. 1999*b*). Therefore the association between fructosamine concentrations and worm numbers is unlikely to be solely due to variation in immune responsiveness although a role for immune responses cannot be entirely ruled out.

Another explanation for the observation that lambs with low fructosamine concentrations acquire more nematodes after anthelmintic treatment is that these lambs graze differently than their contemporaries. For example, lambs with low fructosamine concentrations might graze lush areas in an attempt to restore their relative protein status; as faeces can act as a fertilizer, lusher grass may be more heavily contaminated with nematodes (Hutchings *et al*. 1998, 1999). This could explain the observation that lambs with low fructosamine concentrations acquire more nematodes. Interestingly, if animals with above average levels of parasitism ingest more parasites than average, this would produce the skewed distributions of nematodes commonly observed in grazing sheep.

As fructosamine concentrations are repeatable, moderately heritable and associated with a variety of parasitological variables, they appear to be useful markers to assess the magnitude of natural, predominantly *T. circumcincta* infection. However, the relationship between the severity of infection and fructosamine concentration was relatively complex. Therefore, fructosamine concentrations would be most useful in conjunction with other indicator traits such as egg counts, parasite-specific IgA responses and plasma pepsinogen concentrations.

These results come from a single commercial farm where several management factors made this study particularly powerful. All animals were of known pedigree, from the same breed, born within a short space of time and reared in similar conditions. During the post-weaning period, all animals were kept on the same field. The regular use of anthelmintics made it possible to disentangle the effects of previous, current and future infections. Anthelmintic treatment can reduce infection levels and interfere with the development of immunity to nematodes in sheep (Sutherland *et al*. 1999). The effect of anthelmintic treatment on the development of immunity may differ among nematode species, at least in calves (Claerebout *et al*. 1998) and lambs (Barger, 1988). In this study, comparisons were made within an homogeneous group and are unlikely to be influenced by treatments that were given to all animals. However, more research is necessary before we can make meaningful comparisons among different flocks. In addition, more research is required on the best method to combine the information from fructosamine concentrations with information from different markers.

In conclusion, fructosamine concentrations appear to be a useful indication of the intensity of natural, predominantly *T. circumcincta* infection and they may indicate which lambs are likely to subsequently acquire above-average numbers of this nematode.

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