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## BRIEF COMMUNICATION: Performance of lambs exposed to a desensitising regime prior to infection with *Trichostrongylus colubriformis*

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### Introduction

Enhancing the immune response of lambs against the gastrointestinal nematode *Trichostrongylus colubriformis* through vaccination has been previously investigated (Windon et al. 1984; Douch 1989; McClure 2009). However, the majority of production loss from this parasite species in sheep has been attributed to components of the immune response (Greer et al. 2005). As such, manipulation of the host immune response through desensitisation may provide a novel method of reducing production loss associated with infection. The objective of this investigation was to evaluate the ability of a desensitising regime with parasite antigens to reduce the production cost associated with *Trichostrongylus colubriformis* infection in lambs.

### Materials and methods

All procedures were carried out with approval from the Lincoln University Animal Ethics Committee (Approval No. LU455).

#### Antigen preparation

Soluble somatic antigen was prepared from L<sub>3</sub>, L<sub>4</sub> and L<sub>5</sub> stages of the gastro-intestinal nematode *Trichostrongylus colubriformis*. Immediately following collection, each nematode stage was stored at -20°C until the extraction of crude somatic antigen by homogenisation in ice cold PBS using a ribolyser sample homogenizer and then clarified by centrifugation (14,000 × g for 10 minutes at 4°C). Protein concentrations were estimated using a BCA assay kit (Pierce). Antigen preparations were stored at -20°C until use.

#### Immunization and challenge regime

Twenty-one helminth-free four-month-old Poll Dorset x East Friesian sheep were allocated hierarchically by live weight (mean 33.5 kg ± 0.67 SEM) into one of three groups (n=7). Each group was randomly allocated to one of three treatments. One group (DES) received a desensitising regime that consisted of three weekly injections of 7.5 µg of L<sub>3</sub>, 5.5 µg of L<sub>4</sub> and 10.5 µg of L<sub>5</sub> somatic antigens. The antigen mixture was contained in 200 µl of PBS which was injected into the rectal sub-mucosa at a site approximately 2-3 cm from the recto-anal junction. The remaining two groups (INF and CON) received a

sham injection of 200 µl PBS. One week after the final injection, groups DES and INF were challenged with the equivalent of 80 *T. colubriformis* L<sub>3</sub> larvae per kg live weight per day three times weekly until day 56. The challenge dose was adjusted weekly, based on the mean live weight of all infected animals. The number of required larvae were pipetted onto filter paper which was rolled and administered using a balling gun. Group CON remained as an uninfected control.

#### Measurements and sampling

Animals were housed individually in pens with access to fresh water and a non-mineralised salt block, and were offered lucerne pellets *ad libitum*. The amount of feed offered each day was recorded with the refusals collected and recorded weekly. Subsamples of feed offered and feed refused from each individual were dried at 70°C for a minimum of 72 h for the determination of weekly dry matter (DM) intake. Live weights were recorded weekly. Faecal samples were taken directly from the rectum of each individual weekly. A subsample of the faeces collected was used for the determination of the concentration of nematode eggs in fresh faeces (MAFF 1979). The remainder of the faecal sample was weighed and dried at 70°C for a minimum of 72 h and used to calculate the concentration of nematode eggs per gram of dry faeces. Apparent *in vivo* DM digestibility was measured in an additional eight animals offered the same pelleted lucerne ration housed in metabolism crates during which time DM intake and DM of faeces produced were measured. Voluntary feed intake (kgDM) of the lambs was multiplied by 1 minus the DM digestibility to calculate the DM of faeces produced per day, which was multiplied by the concentration of nematode eggs per gram of dry faeces to give total daily nematode egg excretion. Animal sampling and recording commenced three weeks prior to infection and continued until nine weeks post-infection.

#### Statistical analyses

Data were analysed using Minitab statistical package (Minitab 15, Minitab Inc 2006). Total nematode egg production was log<sub>10</sub>(n+1) transformed prior to analysis for infected groups only from week three of infection and analysed as repeated measures using restricted maximum likelihood (REML). Liveweight gain, voluntary feed intake and feed

conversion efficiency (FCE; g liveweight gain per kgDM consumed) of the three treatment groups during the pre-infection period (weeks -3 to 0), first three weeks of infection, weeks three to six of infection and weeks six to nine of infection were compared using a one-way ANOVA.

## Results and discussion

Desensitising animals to parasite antigens may mitigate the production loss caused by infection with the small intestine nematode *Trichostrongylus colubriformis*. This can be considered a novel approach compared with previous investigations to promote immune development through vaccination (Windon et al. 1984; Douch 1988; McClure 2009). Although the effect was temporary, the desensitising regime imposed upon the DES group appeared to protect animal performance up until six weeks of infection. Overall, the impact of infection alone on liveweight gain and FCE (INF v CON, Table 1) was consistent in magnitude and temporally to previous studies with this nematode species at similar larval challenge rates (Kimambo et al. 1988; Greer et al. 2005). As such, the lack of detrimental effect of the parasite infection during the first three weeks of

prevented by the desensitising regime. Previous investigations with this nematode species have reported a majority of the cost of infection to be a consequence of the nutritional demand imposed by the developing immune response, with infection in immune-suppressed animals having a low level of pathogenicity (Greer et al. 2005). Given that total feed intake during weeks three to six was similar across all groups, the advantage in liveweight gain observed in DES compared with INF animals presumably reflects reduced diversion of nutrients away from productive functions for immune development (Coop & Kyriazakis 1999). With this in mind, this method may be applicable to other nematode species of similar pathogenicity to *T. colubriformis*, although the suitability of a desensitising regime for nematode species that may be considered more pathogenic remains to be determined.

Despite *T. colubriformis* being an intestinal dwelling nematode, the rectal sub-mucosa was a suitable route of administration for the antigen mixture. Delivery of larval antigens to this site in combination with an adjuvant has been shown previously to generate mucosal protection against this nematode (McClure 2009). In the current study no

**Table 1** Mean liveweight gain (kg ± s.e.m), voluntary feed intake (kgDM ± s.e.m) and feed conversion efficiency (FCE; g liveweight gain per kgDM consumed ± s.e.m) during the pre-infection period (weeks -3 to 0 of infection), weeks 0 to 3 of infection, weeks 3 to 6 of infection and weeks 6 to 9 of infection for animals that were either sham-injected uninfected controls (CON), sham injected and infected with the equivalent of 80 L3 *Trichostrongylus colubriformis* larvae per kg live weight per day (INF) or desensitised with sub-mucosal injections of somatic *T. colubriformis* parasite antigens prior to infection with the equivalent of 80 L3 *T. colubriformis* larvae per kg live weight per day (DES). Data represents mean ± SEM.

					Effects (P value)		
		CON	INF	DES	CON v INF	CON v DES	INF v DES
Pre-infection	Liveweight gain (kg)	5.43 ± 0.702	6.14 ± 0.759	5.57 ± 0.695	ns	ns	ns
	Intake (kgDM)	38.9 ± 2.01	38.37 ± 1.56	39.5 ± 1.52	ns	ns	ns
	FCE (g gain per kgDM)	142 ± 18.9	161 ± 20.1	139 ± 14.3	ns	ns	ns
Weeks 0 to 3	Liveweight gain (kg)	5.43 ± 0.971	5.60 ± 0.697	5.54 ± 0.664	ns	ns	ns
	Intake (kgDM)	48.8 ± 1.96	49.8 ± 1.93	48.0 ± 1.08	ns	ns	ns
	FCE (g gain per kgDM)	110 ± 19.0	112 ± 11.5	116 ± 15.1	ns	ns	ns
Weeks 3 to 6	Liveweight gain (kg)	7.31 ± 0.790	3.89 ± 0.809	6.06 ± 0.596	0.01	ns	0.05
	Intake (kgDM)	51.6 ± 3.00	47.2 ± 3.39	51.5 ± 1.38	ns	ns	ns
	FCE (g gain per kgDM)	141 ± 11.8	78 ± 12.8	118 ± 12.3	<0.01	ns	0.04
Weeks 6 to 9	Liveweight gain (kg)	4.16 ± 0.50	-2.44 ± 1.32	-2.87 ± 1.69	<0.01	<0.01	ns
	Intake (kgDM)	55.1 ± 3.42	39.2 ± 3.14	43.1 ± 2.77	<0.01	0.02	ns
	FCE (g gain per kgDM)	76 ± 9.9	-79 ± 39.5	-83 ± 47.8	<0.01	<0.01	ns

infection was anticipated. Compared with CON animals, from weeks three to six of infection INF animals exhibited reductions in liveweight gain and FCE of 47% and 55%, respectively. In comparison, the performance of DES animals was similar to CON animals throughout the first six weeks of infection with 63% of both the loss in liveweight gain and FCE experienced by INF animals during weeks three to six

adjuvant was used, with the delivery of the antigen preparation injected directly into the rectal submucosa appearing to provide an effective desensitising regime. However, such effects were temporary, as indicated by the reduced performance of DES compared with CON animals during weeks six to nine of infection, which coincided with a reduction in total daily nematode egg excretion in DES animals from a peak of 3 million eggs per day at six weeks post-infection to 1.5 million

eggs per day at nine weeks post-infection (data not shown). Although no significant differences in total egg excretion were observed, INF animals did appear to have an earlier peak between weeks four and five of infection. The mechanism of action of the desensitising regime is yet to be elucidated, however, the combined results of animal performance and total nematode egg excretion indicate the desensitising regime may have delayed recognition of the parasite antigen. It remains to be determined if the length of protection afforded by this approach can be extended through alternative antigen delivery techniques. Clearly, further optimisation of the desensitising protocol, including route of administration and exploration of its suitability for other nematode species, is required before this can be considered a practical means of mitigating the impact of gastrointestinal nematode infection in lambs.

In summary, desensitising sheep to *T. colubriformis* antigens may provide a novel method of mitigating the impact of infection with this nematode. However, while this study demonstrates proof of concept, further investigations to optimise and refine the desensitising protocol are required before this can be considered a practical option.

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