Anim. Prod. Aust. 2002 Vol. 24: 113-116

PROTEIN SUPPLEMENTATION BUT NOT ORAL VACCINATION WITH INFECTIVE LARVAE ACCELERATES THE DEVELOPMENT OF RESISTANCE TO *Trichostrongylus colubriformis* OF GRAZING MERINO WEANERS

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SUMMARY

This experiment was designed to test the effectiveness of oral vaccination with infective larvae and protein supplementation in accelerating the development of resistance of grazing Merino weaners (6 months old) to infection with *T. colubriformis*. Animals grazed as a single flock and received 3 times each week either 0 or 140 g/day cottonseed meal (CSM). Within each level of supplementation animals were either repeatedly treated with anthelmintic to preclude infection, exposed to field infection or vaccinated with a total of 42,000 *T. colubriformis* L_3 in 8 doses over an 18 day period. After 7 weeks all infections were terminated and all animals received a challenge infection with 24,000 *T. colubriformis* L_3. Four weeks after challenge worm counts were determined. Sheep that received CSM grew faster (45 g/day) and when exposed to field infection had a FEC 67% lower than nil fed animals. Worm counts after challenge were not affected by vaccination but were 28% lower in animals previously fed CSM. In summary, supplementation with CSM hastened the development of resistance to *T. colubriformis* but vaccination was ineffective.

Keywords: protein supplementation, vaccination, *T. colubriformis*, sheep, nematodiasis, immunity

INTRODUCTION

The failure of young grazing sheep to develop immunity to gastrointestinal (GI) parasites until well into the second year of life represents the major animal health challenge to the Australian sheep grazing industry (McLeod 1995). A number of explanations have been suggested for the slow development of immunity to GI parasites in young sheep at pasture including (i) insufficient antigenic stimulation resulting from rates of field infection insufficient to initiate an effective immune response (Windon *et al.* 1984); (ii) interrupted infections due to anthelmintic treatment which delays or diminishes previously acquired immunity (Barger 1988); and (iii) inadequate supply of scarce nutrients such as metabolisable protein (MP) resulting in an inability to supply the metabolic precursors required by the immune system during parasitic infection (van Houtert *et al.* 1995).

In contrast to the situation with young sheep grazing at pasture, pen-based experiments have demonstrated that immunity to *T. colubriformis* (Barnes and Dobson 1993) can be acquired by young sheep following just weeks or months of infection. Nevertheless, susceptibility of young grazing sheep to GI parasites remains an industry problem and differences between pen and field observations have yet to be adequately explained. Therefore, this experiment was designed to test the following hypotheses. Firstly that the prolonged susceptibility of young Merino sheep grazing at pasture to GI parasites was due to insufficient antigenic stimulation and that oral vaccination with GI parasites would accelerate the development of resistance. Secondly that an increased supply of MP would further enhance the beneficial immunological effects of vaccination and provide complete resilience to infection. Resilience refers to the ability of animals to maintain production during infection.

MATERIALS AND METHODS

Experimental design, animals and location.

The experimental design was factorial with two levels of supplementation and three levels of nematode infection. Sixty eight finewool Merino wether weaners (6 months of age) that had been born and raised on pasture were used in the experiment. Animals were stratified on the basis of initial live weight and allocated randomly from within each live weight group to infection and supplementation regimen. The mean (± sd) live weight of the animals at the start of the experiment was 20.9 ± 2.1 kg and at this time animals were drenched with Numectin® to remove GI nematodes (Ivermectin, 200 µg per kg; Nufarm Animal Health). Animals grazed as a single group in the

experimental paddocks (total area of 5 ha) at the University of New England, Armidale. The experiment was approved by the Animal Ethics Committee of the University of New England.

Supplementary feed and feeding protocol

Pelleted CSM (900 g DM/kg fresh weight; 920 g OM/kg DM; 300 g CP/kg DM; 10 g P/kg DM) was used as the supplementary feed. Typically CSM contains *c.* 50% rumen degradable protein and is expected to supply 150 g MP/kg DM (Freer et al. 1997). From day 1-49 supplemented animals (n = 34) were offered CSM on 3 days each week at a level to provide for a daily offering of 140 g/head. The feeding protocol was such that all animals were mustered to yards where supplemented animals were separated and offered the CSM in individual pens (dimension 1.0 x 0.3 m). Animals were given 45 min to consume the supplement and then residual supplement was weighed and subsampled. Animals that did not receive supplement (n = 34) were held in a yard during this period.

Infection details

Within each supplement regimen, animals were subjected to three different infection regimens namely, suppresively drenched (D; n = 8), field infection (FI; n = 13) or FI plus artificial infection (AI; n = 13). Animals in the D group were given an oral drench with Cydectin® (moxidectin, 0.2 mg/kg; American Cyanamid Company) and Scanda® (8 mg/kg levamisole hydrochloride and 4.5 mg/kg oxfendazole; Schering-Plough Animal Health) on day 1. On day 14 and thereafter weekly for the following 5 weeks, D animals were drenched with Numectin® and Scanda®. Animals in the AI group were given an artificial infection of 42,000 *T. colubriformis* L_3 over an 18 day period. The artificial infection consisted of 15,000 L_3 on days 1 & 18; and 2,000 L_3 on days 3, 5, 7, 10, 12 and 14. On day 49 all animals were drenched with Numectin® and Scanda ® and CSM supplementation ceased. On day 56 a total of 32 animals comprising 12 from each of the FI and AI groups and 8 from the D group were selected at random and challenged with 24,000 *T. colubriformis* L_3. The remaining animals (n = 36) did not receive further artificial infection. On day 84, animals that had been given an artificial challenge were stunned with a captive bolt gun and then exsanguinated.

Animal and pasture sampling

Animals were weighed weekly between days 1 - 56 and then again at days 77 and 84. On the same days faeces were collected to estimate faecal egg count (FEC). On day 42, faeces were bulked within treatment groups and cultured for 7 days to determine nematode genera contributing to FEC. After slaughter (day 84), the small intestine was immediately recovered and processed for estimation of number of adult males, adult females, juveniles and by addition of these parameters the total number of intestinal nematodes of the various genera. Pasture was sampled to determine herbage mass (kg DM/ha) and percentage green and dead on days 3, 42 and 83. The sampled herbage was dried (80°C for 5 days), ground (1 mm) and analysed to determine N (presented as crude protein (CP) = N x 6.25 g/kg DM) and phosphorus (P; g/kg DM).

Statistical analysis

A Generalised Linear Model was used in the analysis of data fitting the independent effects of supplementation and infection. Repeated measures was used for all variables except worm counts. Starting live weight of animals was used as a covariate for the analysis of live weight. Faecal egg count and *T. colubriformis* numbers were cuberoot transformed prior to analysis and backtransformed means are presented in this paper. In all other cases least squares means are used throughout the results. The computer program SAS (SAS Institute Inc. 1990) was used for all analyses.

RESULTS

Mean values for total and green herbage mass (kg DM/ha), CP (g/kg DM) and P (g/kg DM) were 2,461, 1,360, 100 and 2.1 respectively on day 3; 3268, 608, 67 and 1.9 respectively on day 42; and 1856, 145, 86 and 1.6 respectively on day 83. Average intake (± sd) of CSM was 134 ± 3.2 g/day. Supplementation with CSM increased (week 1 $P<0.05$; weeks 2-7 $P<0.01$) live weight during the initial 7 week infection period with differences apparent 1 week after the start of feeding (Figure 1). Mean weight gain over the first 7 weeks for supplemented animals was 102 g/day which was greater ($P<0.01$) than the 57 g/day recorded for nil fed animals. Weight gain during the post challenge period (day 56-84) was unaffected by previous feeding regimen so that live weight of previously fed animals continued to be greater ($P<0.01$) (Figure 1). Live weight was unaffected by infection regimen and there was no significant interaction between the effects of supplementation and infection.

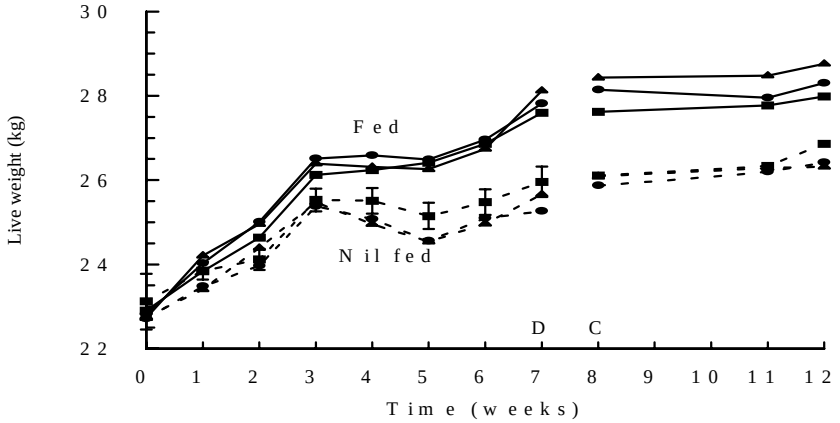

Figure 1. Live weight (mean ± pooled SEM) during the initial infection period (weeks 1-7) and following challenge with 24,000 *T colubriformis* L_3 (weeks 8-12). Animals were supplemented with cottonseed meal (Fed) or not (Nil fed) and were either drenched weekly (▲), or received field infection (●) or field plus artificial infection with *T colubriformis* L_3 (■). D represents drenching and C the time of challenge.

Faecal egg count of FI animals was reduced (P<0.05) by CSM when averaged over the initial 7 week period (Figure 2). Supplementation did not affect FEC of animals which received AI. Faecal egg count 3 and 4 weeks post challenge (weeks 11 and 12) was unaffected by prior infection or supplementation regimen and averaged 566 epg and 898 epg respectively. Larval culture of faeces collected at day 42 indicated that 67% and 78% of nematode eggs were *Trichostrongylus* spp in FI and AI weaners respectively. *Teladorsagia* spp accounted for, on average, 16% and 14% and *Haemonchus contortus* 16% and 8% of nematode eggs in FI and AI animals respectively.

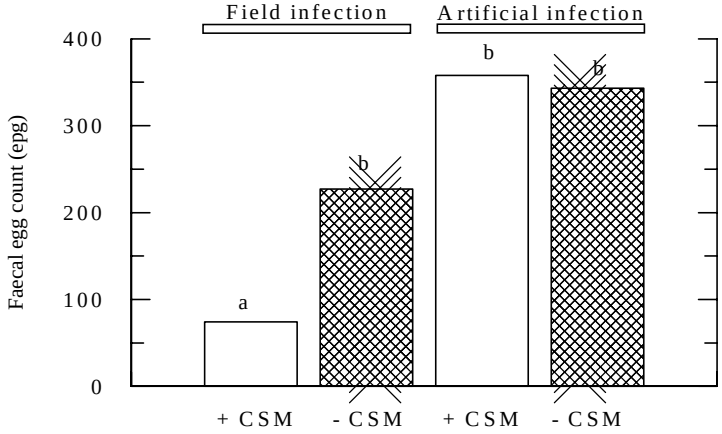

Figure 2. Backtransformed mean faecal egg count during the initial infection period (weeks 1-7). Animals were either supplemented with cottonseed meal (+ CSM) or nil fed (- CSM) and received either field infection or field plus artificial infection.

Counts of adult and juvenile *T. colubriformis* on day 84 were unaffected by previous infection regimen or supplementation and total adult and juvenile counts averaged 9,367 and 182 respectively. However, there was an indication (P=0.27) that animals which had previously received CSM had fewer adult *T. colubriformis* after challenge. Mean total adult counts for CSM supplemented and nil fed groups were 7,739 and 10,750 respectively.

DISCUSSION

The hypotheses tested in this experiment were twofold. Firstly, oral vaccination, achieved by AI, of young grazing Merino weaners would accelerate the development of resistance. Secondly, that an increased supply of MP would further hasten the beneficial immunological effects of vaccination and provide complete resilience to infection. Our findings do not support the first hypothesis. Artificial infection with 42,000 *T. colubriformis* L_3 administered over an 18-day period and resident in the animal for a further 31 days did not result in greater resistance to a subsequent challenge infection.

The failure to increase the immunocompetence of young grazing sheep with this vaccination protocol is in contrast to that observed with similar, but housed, animals. For example Barnes and Dobson (1993) infected Merino lambs (26 weeks of age) with 36,000 *T. colubriformis* L_3 over a 6-week period and reported establishment rates to subsequent challenge of 9% (omitting 1 non-responder) indicating the development and persistence of resistance to infection. Similarly Windon et al. (1984) demonstrated that vaccination with 2 x 20,000 irradiated *T. colubriformis* L_3 over a 4-week period provided 76% protection to subsequent challenge. In the current experiment however, establishment rates to subsequent challenge were considerably greater at around 39%. This figure is an estimate only because field infection following artificial challenge could not be quantified.

The lack of an effective immunological response by young grazing sheep to vaccination procedures similar to those successful in housed animals is difficult to reconcile but is an important finding of this experiment. It is possible that differences between grazing and housed sheep to vaccination are due to dietary insufficiencies experienced by grazing sheep but not by housed sheep because of the routine addition of mineral and vitamin premixes to experimental foods. For example, dietary levels of phosphorus (Coop and Field 1983), molybdenum (McClure et al. 1999) and cobalt (Suttle and Jones 1989) have been reported to influence the acquisition of protective immunity.

In regards to the second hypothesis, supplementation with CSM increased resistance to field infection, reduced *T. colubriformis* burden by 28% following challenge but did not lower FEC of AI animals. It is possible that the increased MP supply as a result of CSM supplementation accelerated the acquisition of resistance resulting in reduced establishment rates of incoming larvae in FI animals. Such effects were not evident in AI animals where 21,000 L_3 were administered by day 7 at which stage effects on establishment may not yet have been fully operational. Dietary effects on establishment of *T. colubriformis* have been reported in periparturient ewes (Donaldson et al. 2001) but in contrast, van Houtert et al. (1995) concluded that MP supply was ineffective at altering establishment rates of *T. colubriformi*s in young Merino sheep although it enhanced immunity against mature worms. An alternative explanation to account for the effect of CSM supplementation in reducing FEC of FI but not AI animals is that CSM reduced pasture intake and hence larval intake. However, as FEC of FI animals was reduced by 77% and pasture substitution, as a result of CSM supplementation, estimated (Freer et al. 1997) to be only 6%, this explanation is unlikely to have been a major factor in the reduced FEC of FI animals. Supplementation with CSM increased weight gain but as infection regimen did not influence growth it was not possible to assess effects on resilience.

In conclusion, our results support the usefulness of CSM supplementation in accelerating the development of resistance to *T. colubriformis*. On the other hand, oral vaccination with live *T. colubriformis* L_3 had no such effect. The differences between grazing and housed animals in immunoresponsiveness to vaccination are important and warrant further consideration. Identification of the factors responsible for these differences will be of great value to sheep producers dealing with increasing levels of anthelmintic resistance.

ACKNOWLEDGEMENTS

We acknowledge the Australian Centre for International Agricultural Research project no. 9422 for financial assistance.

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