Livestock Science 157 (2013) 568–576

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Immune response of twin-bearing Merino ewes when infected with *Haemonchus contortus*: Effects of fat score and prepartum supplementation

F.A. Macarthur [a], L.P. Kahn [a,*], R.G. Windon [b]

[a] School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia
[b] Animal, Food and Health Sciences, CSIRO, F.D. McMaster Laboratory, Locked Bag 1, Armidale NSW 2350, Australia

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form
23 August 2013
Accepted 23 August 2013

Keywords:
Immunity
Haemonchus contortus
Body composition
Protein supplementation
Periparturient ewes
Leptin

ABSTRACT

The benefit of providing prepartum protein supplementation to twin-bearing Merino ewes with low and high fat score (FS; measure of soft tissue depth related to fatness) was investigated to quantify effects on host resilience and immunity to *Haemonchus contortus*. The experiment was conducted on low quality pastures from day (d) -91 to weaning at d 52, relative to the mid-point of lambing (d 0). The experimental design consisted of two feeding regimes designed to achieve FS targets of 2 (low) and 4 (high) by d -47 followed by allocation to protein supplementation groups (0 or 200 g/d cottonseed meal; CSM) until d 10. Throughout the experiment there were two infection groups (0 or 750 *H. contortus* third stage larvae (L_3)/week). Peri and postparturient ewes were susceptible to *H. contortus* shown as a rise in worm egg count (WEC). High FS group ewes had a significant benefit in terms of protection against *H. contortus*, but this was restricted to the prepartum period. There were no benefits of supplemental protein which did not enhance protective immunity. Lower prepartum WEC from high FS ewes was associated with higher levels of circulating eosinophils but a general decline in counts occurred prior to parturition. Infected ewes had elevated antibody titres and a greater number of mast cells in abomasal tissue at slaughter on d 52. The results suggest that sufficient fat and protein reserves are important for twin-bearing Merino ewes during pregnancy to elevate the immune response against *H. contortus* whereas strategic prepartum protein supplementation had no benefit.

1. Introduction

Gastrointestinal nematode (GIN) parasites are a significant economic and welfare constraint to the Australian sheep industry (Sackett et al., 2006; Kelly, 2011). *Haemonchus contortus* is the most important GIN in the summer rainfall zone of Australia and is distinguished as a blood sucking parasite with high fecundity. Under favourable environmental conditions, there can be significant levels of production loss and mortality as a result of haemonchosis (Kelly et al., 2010). Periparturient ewes have increased susceptibility to GIN infections due to a temporary loss of immunity during parturition and lactation which is manifested as a rise in worm egg count (WEC) commonly referred to as the periparturient rise (PPR) (Taylor, 1935; Gibbs, 1982).

Supply of metabolisable protein (MP) plays a role in reducing the extent of the PPR and aids in increasing the performance of ewes with GIN infection (Donaldson et al., 1997; Houdijk et al., 2000; Kahn et al., 2003; Valderrabano et al., 2006); however, its benefit to periparturient ewes infected with *H. contortus* remains less certain. The provision of MP is most effective when its supply is scarce and

* Corresponding author. Tel.: +61 2 67732997.
E-mail address: lkahn3@une.edu.au (L.P. Kahn).

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http://dx.doi.org/10.1016/j.livsci.2013.08.017

demand high as in the periparturient ewe, particularly when grazing a low-quality pasture. Immunity of periparturient ewes can also be enhanced through MP supplied by catabolism of body muscle (Houdijk et al., 2001) and therefore ewes with lower fat score (FS; a measure of fat and muscle depth at the GR (Greville) site; Holst and White, 2001; Shands et al., 2009) should be more likely to show benefits for host resilience and immunity to GIN from supplemental MP.

A companion publication (Macarthur et al., 2013) describes the interaction between prepartum protein supplementation and the fat and protein reserves of Merino ewes in regulating maternal production when grazing low quality pastures. In this paper, we quantify the effect of FS and prepartum protein supplementation on host resilience and immunity to *H. contortus* of twin-bearing Merino ewes. The specific hypothesis was that supplementation would be most effective in low FS ewes when they are unable to meet their nutrient requirements from tissue catabolism or from pasture.

2. Materials and methods

Full details of the experimental design, supplementation and infection details have been described previously (Macarthur et al., 2013). The timing of all experimental events is provided in relation to the mid-point of lambing (day; d 0). The experiment was carried out with the approval of the Animal Ethics Committee of the University of New England (AEC 06/052).

2.1. Experimental design

Briefly, the $2 \times 2 \times 2$ factorial experiment was undertaken on the Northern Tablelands of New South Wales, Australia, with two levels of periparturient nutrition designed to achieve FS targets of 2 (low) and 4 (high), representing below and above the Australian industry target of 3, two infection groups (0 or 750 *H. contortus* third stage larvae (L_3)/week) and two prepartum protein supplementation groups (0 or 200 g/d cottonseed meal (CSM); 89.2% DM; 482 g CP/kg DM; *ca* 45% rumen undegradable dietary protein) instigated following the formation of FS groups. Merino ewes of mixed age (2–5 yr) were naturally mated to Merino rams and ultrasound scanned at d -91 to identify twin-bearing ewes. Ninety six twin-bearing ewes were then ranked on FS and allocated to infection and FS group such that the mean FS of each group was equivalent. Low and high FS groups were generated in an animal house from d -91 to -47 after which ewes were reallocated to supplement group, removed from the animal house and grazed on pasture. Ewes were rotated weekly among 4×4 ha paddocks comprised of low quality naturalised pastures and were supplemented or not until d -10. At the completion of the supplementation period, ewes were randomly allocated from within infection group on the basis of FS and supplement group to form 4 lambing groups (i.e. 2 uninfected and 2 infected groups) until weaning at d 52. Ewes and their lambs were monitored for a range of traits from d -91 to weaning at d 52 and ewes were killed at d 52 for post-mortem sampling.

2.2. Anthelmintic and infection details

All ewes received a quarantine drench at pregnancy scanning (d -91) with abamectin and albendazole at recommended dose rates. In addition to the quarantine drench, uninfected ewes were given an ivermectin controlled-release capsule (160 mg ivermectin/capsule; Merial, Australia) and drenched with levamisole and napthalophos at recommended dose rates. Faecal worm egg counts (WEC) were performed on d -81 to confirm negative WEC. Uninfected ewes also received fortnightly treatments of abamectin throughout the experimental period to minimise the chance of reinfection. Infected ewes received an oral dose of 750 *H. contortus* L_3/wk [Kirby strain, susceptible to all anthelmintics; (Le Jambre et al., 2008)], provided as 3 equal doses given on Monday, Wednesday and Friday. Infection started on d -84 and continued throughout the experiment. On d -47 all ewes received abamectin and levamisole at recommended dose rates because of concern of clinical effects of infection on infected low FS ewes.

2.3. Clostridial vaccination

Ewes were vaccinated subcutaneously (1 mL; Ultravac™5 in 1, Pfizer Animal Health, Australia) against clostridial diseases on days -47 and -14. The vaccination contained toxins for prevention of the five main clostridial diseases in sheep: *Clostridium perfringens* type D; *C. tetani; C. novyi* type B; *C. septicum* (as ultrafiltered toxoids) and *C. Chauvoei* (as formol culture).

2.4. Worm egg count and larval differentiation

Worm egg counts were conducted weekly to monitor parasitic infections. The samples were analysed with a modified McMaster technique (Whitlock, 1948) to estimate eggs per gram (epg) of faeces for each animal. Eggs were counted under $10\times$ objective with 1 egg equivalent to 60 epg of faeces. Faeces from each animal were pooled within treatment groups, mixed with vermiculite and cultured at 25 °C for 7 d. The resulting L_3 were stained with Lugol's iodine solution (1% iodine, 2% potassium iodine), and 100 L_3 were examined at $400\times$ magnification to determine the proportion of each parasite genera present.

2.5. Post-mortem processing

Ewes from each treatment group were killed by captive bolt followed by exsanguination on d 52. The abomasum and small intestine were immediately recovered and processed for differential worm counts, histological determination and local (tissue) immunoglobulin responses.

The abomasum and approximately the first 10 m of small intestine were removed from the gastrointestinal tract. The organs were processed separately by opening via incision, thoroughly washed with warm water, mucosal surfaces scraped and the washings collected. The clean abomasum was digested in 200 mL 1.7% HCl to remove inhibited larvae and the digest combined with abomasal washings. All volumes were adjusted to 2 L and four representative