Veterinary Parasitology 188 (2012) 306–324

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

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

# The influence of reproductive physiology and nutrient supply on the periparturient relaxation of immunity to the gastrointestinal nematode *Trichostrongylus colubriformis* in Merino ewes

A.M. Beasley[a,b,*], L.P. Kahn[b], R.G. Windon[c]

[a] *Australian Sheep Industry CRC, CJ Hawkins Homestead, University of New England, Armidale, NSW 2350, Australia*
[b] *Centre for Animal Health and Welfare, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia*
[c] *CSIRO Livestock Industries, F.D. McMaster Laboratory, Locked Bag, Armidale, NSW 2350, Australia*

ARTICLE INFO

*Article history:*
Received 23 January 2011
Received in revised form 13 March 2012
Accepted 14 March 2012

*Keywords:*
Periparturient
*Trichostrongylus colubriformis*
Nutrition
Body composition
Leptin
Early weaning
Lactation
Physiology
Immunity

ABSTRACT

A pen experiment was conducted to investigate the interaction of early-weaning and nutrient supply on the periparturient relaxation of immunity to the gastrointestinal nematode (GIN) *Trichostrongylus colubriformis* in Merino ewes. Mixed-age pregnant and non-pregnant (dry) ewes were infected with 8,000 *T. colubriformis* $L_3$/week, and fed either a high or low quality diet. Following parturition, lambs were either removed from their mothers at 2 days of age or allowed to continue suckling. Systemic immunity began to wane during late pregnancy with circulating eosinophils and plasma total antibody (Ab) levels declining from day −37 (relative to the midpoint of lambing) and day −24, respectively. Pregnant ewes fed the low quality diet exhibited an increasing faecal worm egg count (WEC) from day −24 and had higher intestinal worm burdens on day 13, whereas ewes fed the high quality diet had a delayed transient rise in WEC of lower magnitude. Dry and early-weaned ewes remained highly resistant to *T. colubriformis* at all times. In the post-lambing/lactation period, ewes fed the high quality diet had higher levels of local total Ab and numbers of goblet cells (GC) in the small intestine on days 13 and 41. Lactating/suckled ewes had a lower anti-parasite local immune response as indicated by reduced titres of total Ab, $IgG_1$, IgM and IgA and lower numbers of mucosal mast cells (MMC), globule leukocytes (GL) and GC in small intestinal tissue compared to their dry and early-weaned counterparts. Early-weaning resulted in rapid recovery of blood eosinophils and total Ab. On day 13 post-lambing, titres of total Ab, $IgG_1$, IgM, IgA and IgE, and numbers of MMC and GL were greater than those measured in dry and suckled ewes. When fed the high quality diet, ewes had a higher dry matter (DM) intake, maternal weight, fat score, greater fat depth and eye muscle depth, birthed heavier lambs that had higher growth rates, and produced more milk. The physiological status of pregnancy resulted in a higher DM intake but lower measures of fat depth and eye muscle depth, and suckling led to an increase in DM intake but a reduction in body weight and fat score through mobilisation of fat and muscle reserves. Despite the marked effect of diet quality on production traits, some inconsistencies were observed between body composition and apparent parasite resistance, measured by WEC and worm counts, suggesting that the nutritional influence was not necessarily always mediated through changes in body composition. Although reproductive status affected blood leptin levels, diet had no effect within suckled ewes and therefore it was concluded that leptin has no causative role in maintaining the periparturient relaxation of immunity to *T. colubriformis*.



* Corresponding author at: Centre for Animal Health and Welfare, School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia. Tel.: +61 7 5466 1340.
*E-mail address:* beasleyab@bigpond.com (A.M. Beasley).

0304-4017/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2012.03.022

## 1. Introduction

One of the key factors determining the ability of sheep to mount an effective immune response to gastrointestinal nematode (GIN) infection is host nutrition, and the topic has been extensively reviewed (Coop and Holmes, 1996; van Houtert and Sykes, 1996; Coop and Kyriazakis, 1999; Knox, 2000). Specifically, in the case of the breeding ewe, increased nutrient requirements during late pregnancy and lactation (Kahn et al., 1999; Adams and Liu, 2003; Kahn et al., 2003b; Liu et al., 2003) coupled with the often poor quality and quantity of pasture during those crucial months typically results in a severe shortfall of both protein and energy requirements. The inability of the ewe to meet nutritional requirements exacerbates the periparturient rise (PPR) in faecal worm egg counts (WEC) (Walkden-Brown and Kahn, 2002). A number of studies have demonstrated that the magnitude of the PPR can be regulated by the dietary supply of metabolisable protein (MP) (Donaldson et al., 1998) and also by the mobilisation of tissue reserves (Houdijk et al., 2001a; Valderrabano and Uriarte, 2003). Nevertheless, the precise mechanisms underlying the nutritional basis for the breakdown in immunity in breeding ewes remain unclear. In recent times, the hormone leptin has provided an important link between the nutritional environment of animals and their immune competence. Not surprisingly, a complicit role for leptin in maintenance of the periparturient relaxation of immunity (PPRI) has been hypothesised, but is as yet to be proved.

The aim of this experiment was to characterise the PPRI to GIN infection of sheep in response to two known potent regulators of the PPR, namely lactation and nutrient supply (O'Sullivan and Donald, 1970; Donaldson et al., 1998). Experimental contrasts involving reproductive physiology (dry, early weaned or suckled) and nutrition (high or low quality diet) were compared in order to examine their influence on the parasitological, immunological, endocrine and production responses associated with the PPR. It was therefore hypothesised that this study would more clearly identify the causative mechanisms that predispose the periparturient ewe to an impaired immune response.

## 2. Materials and methods

### 2.1. Animals and housing

The timing of all experimental events is made relative to the midpoint of lambing (day 0) which was 10th November 2005. The mixed-age, fine wool Merino ewes in this experiment were sourced from the University of New England's Kirby station flock. Thirty one ewes were selected at random to remain un-mated and the remaining ewes subjected to oestrous synchronisation using CIDRs (Eazi-Breed CIDR Sheep and Goat Device; Pfizer Animal Health, Australia), and then naturally mated with Merino rams for 3 weeks. On day −99, ewes were ultrasound scanned to identify 52 single-bearing ewes that would lamb within a 2 week period. All 83 experimental animals were shorn after scanning, and following a 10 day acclimatisation period in group pens, housed in individual pens on day −50 with a natural light regimen and continuous access to water. All experimental procedures were approved by the University of New England Animal Ethics Committee (Approval number AEC 05/1434).

### 2.2. Experimental design

Experimental ewes were stratified within pregnancy status (pregnant or non-pregnant, hereafter referred to as "dry") on the basis of live weight, fat score (FS) and WEC measured on day −60, and then allocated at random to treatment group and pen. The experiment consisted of a $2 \times 2 \times 2$ incomplete factorial design with two levels of pregnancy (pregnant or dry), two levels of diet quality (high or low) and, following lambing, two levels of lactation (early-weaned or suckled) (Fig. 1).

The duration of the experiment was 92 days, from day −50 to day 42. All ewes lambed within 10 days. As ewes lambed, they were allocated alternately to either the early-weaned or suckled treatment groups. Those that entered the early-weaned group had their lambs removed 2 days after parturition. Those allocated to the suckled group were allowed to continue suckling their lambs. Randomly selected representatives from each treatment group were killed on days 13 ($n = 41$) and 42 ($n = 42$) for post-mortem sampling.

### 2.3. Infection details

Ewes were drenched with Rycomectin® (abamectin; 0.25 mL/kg body weight; Novartis) and Combi® (albendazole oxide plus levamisole hydrochloride; 0.1 mL/kg body weight; Novartis) on day −60 to remove any existing worm burden acquired from previous grazing. This was confirmed by zero WECs 10 days later. Beginning on day −50, all ewes received a weekly dose of 8,000 *T. colubriformis* infective larvae (McMaster strain, Windon, 1996) administered *per os* in 2 doses of 4,000 $L_3$ suspended in water on Tuesdays and Fridays and this continued for the entire experiment.

### 2.4. Animal sampling and sample processing

Faeces were collected weekly, beginning on day −30, from the rectum of individual ewes to estimate the number of eggs per gram (epg) in fresh faeces using a modification of the McMaster technique (lower level of detection 60 epg). Ewes were weighed fortnightly from day −45 to −17, then weekly until day 40. Lambs were weighed at birth and then on days 18 and 32 after the midpoint of lambing. Fat score, a subjective measure of soft tissue depth at the "GR" site located 110 mm from the midline over the 12th rib (White and Holst, 2006), was recorded fortnightly from day −43 until the end of the experiment. This method was adapted from the procedure described by Jefferies (1961). At days −16, 9 and 32, ewes were ultrasound scanned by an industry accredited operator to measure fat depth (FD) and eye muscle depth (EMD) at the C site.

Blood was collected by jugular venipuncture ($K_3$EDTA vacutainers, Becton Dickinson, Australia). A range of parameters was measured in whole blood or plasma.