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

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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* L₃/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, IgG₁, 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, IgG₁, 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*.

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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 L₃ 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 (K₃EDTA vacutainers, Becton Dickinson, Australia). A range of parameters was measured in whole blood or plasma.

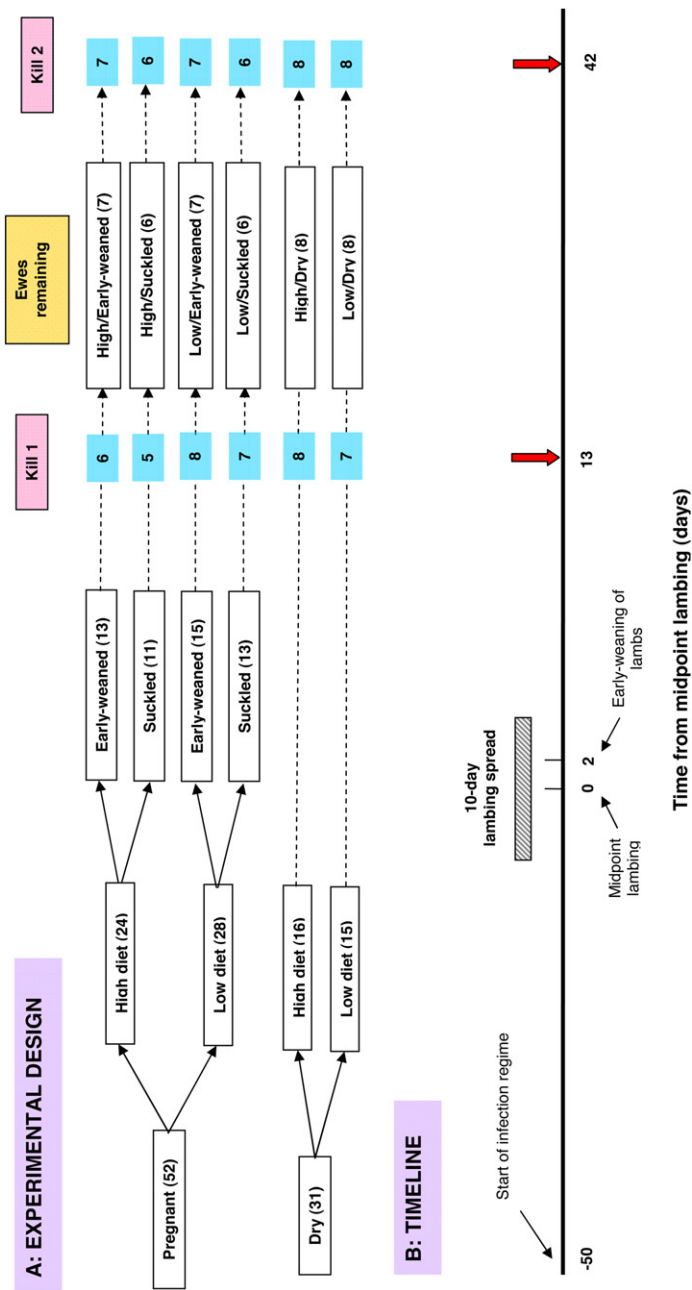


Fig. 1. Experimental design and timeline of events relative to the midpoint of lambing which is defined as day 0 (not to scale). Animals in each group are in parentheses and in shaded boxes.

Plasma was obtained by centrifugation of blood samples at 2000 rpm (721 g) for 20 min, and stored at -20°C until analysis. Circulating antibody levels were measured fortnightly between days -50 and -23 , then weekly for the remainder of the experiment. For measurement of haematological parameters (including differential white cell counts) and blood hormones, blood was collected from individual ewes fortnightly from day -50 to day -23 , then weekly until day -9 . Frequency of sampling increased to 3 times weekly from day -9 to 9, twice weekly from day 12 to 16, then weekly until day 40.

A colostrum sample (approximately 10 mL) was collected from lambing ewes within 12 h of birth. Thereafter, a milk sample (10–15 mL) was collected weekly from suckled ewes for the remainder of the experimental period. Samples were collected into 15 mL centrifuge tubes (Sarstedt Australia Pty Ltd, SA, Australia) and stored on ice until transfer back to the laboratory, then centrifuged at 2000 rpm (721 g) for 20 min at 4°C to separate the fat layer. The whey component was then transferred into 2 mL aliquots (as for plasma) for storage at -20°C until assayed. The method described by Robinson et al. (1969) was used to estimate milk yield of ewes using body weight data on the suckling lamb. The regression equation used was:

$$M = 38.27W + 0.009I^2 + 411$$

where M = the daily milk consumption of the lamb (g/d); W = body weight of lamb (kg), and I = body weight change of lamb (g/d).

The estimation of milk production using this equation is based on nil food intake, other than milk, for lambs and so feed bins were elevated sufficiently to prevent lambs from consuming the ewes' feed ration.

Ewes were killed by captive bolt followed by exsanguination on days 13 and 42. After slaughter, the small intestine was immediately recovered and processed for differential worm counts (WC), histological determination of mast cell (MMC), globule leukocyte (GL) and goblet cell (GC) numbers and local antibody responses as described previously by Beasley et al. (2010b). For the determination of antibody responses in small intestine tissue, a 7–10 cm section approximately 30 cm from the pylorus was removed post-mortem and stored at -20°C . Tissue was homogenised using procedures adapted from Bendixsen et al. (2004). The frozen tissue sections were homogenised with 5 mL of ice cold PBS per gram of tissue and 50 μL of diluted Protease Inhibitor Cocktail (Sigma-Aldrich, NSW, Australia, diluted 1 g in 10 mL deionised H_2O) per gram of tissue. The tissue homogenate was centrifuged at 1,500 rpm ($405 \times g$) for 5 min at 4°C and the supernatant stored at -20°C until assayed.

2.5. Intestinal worm counts and tissue histology

The procedures used for WCs, and counts of tissue MMC, GL and GC have been described previously (Beasley et al., 2010b).

Table 1

Composition of the low and high quality diets fed to Merino ewes throughout the experiment.

	Low	High
Proportion of ration (%)		
Oaten hay	73.0	42.5
Lucerne hay	0.0	42.5
Cottonseed meal	2.0	15.0
Sorghum stubble	25.0	0.0
Diet analysis		
Dry Matter (%)	88.0	88.0
Crude Protein (% DM)	10.8	20.2
Metabolisable Energy (MJ/kg DM)	8.5	9.6
Metabolisable Protein (g/kg DM)*	47.1	79.5

* Average metabolisable protein values estimated for dry ewes (Freer et al., 1997).

2.6. Differential white blood cell counts

Differential white blood cell counts were obtained using the CELL-DYN 3500R automated haematology analyser (Abbott Diagnostics, USA).

2.7. Antibody responses

Total antibody (Ab) in plasma, and total Ab IgG₁, IgG₂, IgM, IgA and IgE in tissue homogenates, specific to *T. colubriformis* L₃, in tissue homogenates, and total Ab in plasma, were determined using an enzyme linked immunosorbent assay (ELISA) previously described (Beasley et al., 2010b).

2.8. Circulating leptin concentration

Measurement of circulating leptin concentrations in ewe plasma was conducted at the School of Animal Biology, University of Western Australia. Samples were assayed for leptin using the double-antibody RIA method of Blache et al. (2000). The limit of detection for leptin in plasma was 0.08 ng/mL and the inter-assay coefficient of variation was <5%.

2.9. Diet and feeding

Chaffed oaten and lucerne hay, cottonseed meal and sorghum stubble were fed in varying proportions to formulate the high and low quality diets which were fed throughout the experiment (Table 1). On day -50 , ewes were offered 1,000 g of feed (fresh weight). Feed refusals were removed and weighed each morning prior to the fresh ration being offered. Three times per week on Monday, Wednesday and Friday, feed offered to individual ewes was re-calculated by averaging feed intake for the previous two days (or 3 days in the case of the weekend) and adding an additional 100 g. Twice weekly, a sub sample (10%) of the feed refused by each animal was collected, bulked and stored at -20°C for dry matter (DM) determination. The diets were estimated to provide MP and metabolisable energy (ME) at 50–70% (low diet) and 80–100% (high diet) of requirements (Kahn, 2003) with greatest deficit occurring post partum.

2.10. Statistical analysis and calculations

Statistical analysis of the data was conducted in three discrete periods to avoid confounding time with physiological status. These periods were P1, prior to lambing (days –51 to –10); P2, during the lambing period (days –7 to 11); and P3, during the postpartum lactation period (days 15 to 42). The main effects of infection status (uninfected or infected), pregnancy (dry or pregnant) and lactation (dry, early-weaned or suckled) were examined by the use of general linear models (SAS Institute, 2006). All two-way interactions were fitted in each model and effects that were not statistically significant were removed. Repeated measures analysis was used when multiple samples were taken from an individual animal within experimental periods. Variables that were not normally distributed were subjected to transformation prior to analysis. The effectiveness of each transformation was confirmed on the transformed data and on the model residuals by reference to the Shapiro-Wilks statistic. Back-transformed least square means $\pm$ 68% confidence intervals (c.i.) are presented in the results. These confidence intervals give a close approximation to standard errors which cannot be back-transformed as they are asymmetrical about the mean. In the case of datasets that did not require transformation prior to statistical analysis, least square means $\pm$ standard errors (s.e.) are presented. Pearson's correlation coefficients represented regressions between pairs of variables measured. Correlation coefficients for the regression between variables were calculated for P2 and P3 only, since no post-mortem data was collected during P1. Animals from all treatment groups were pooled for this purpose. For variables with repeated time points, correlations were performed using the average value for the relevant period. For variables measured at slaughter (e.g. WC, tissue antibody levels), correlations were performed using a single time point. In order to control the Type I error rate arising from the calculation of multiple comparisons (i.e. correlations), the Holm-Bonferroni correction was applied (Quinn and Keough, 2002). Using this correction for the nominated significance level of $P < 0.05$ for 132 comparisons the equivalent significance level becomes $P < 0.0004$ for each individual correlation. This correction is very conservative thereby reducing false positives but it may also increase the incidence of false negatives. Maternal ewe body weights were estimated as actual live weight less the sum of the predicted weights of conceptus and greasy fleece using Grazfeed (Freer et al., 1997). Probability (P) values < 0.05 were considered to be significant.

3. Results

3.1. Faecal worm egg counts

WEC of all treatment groups remained < 400 epg (raw data) throughout the experiment with the exception of suckled ewes fed the low quality diet. This group began to have significantly higher WEC than all other treatment groups from day 18, reaching a maximum of 1,790 epg (raw data) on day 38.

Pregnant ewes had significantly higher WEC on days –24 and –17 (P1) (Table 2) than their dry counterparts ($P = 0.004$) although absolute values of WEC remained very low. During P2, weaning status had a significant effect on WEC ($P < 0.001$) with WEC of lambed ewes being greater ($P < 0.001$) than for dry groups. Weaning status continued to be a significant contributor to differences in WEC during P3 with suckled ewes having higher counts than their early-weaned counterparts at all sample times (793 epg vs. 60 epg respectively; $P < 0.001$, raw values).

Diet had a significant effect on WEC during P2 ($P = 0.021$) and P3 ($P = 0.025$), with ewes fed the low quality diet having higher WEC (108 epg and 351 epg for P2 and P3 respectively) than those fed the high quality diet (46 epg and 50 epg for P2 and P3 respectively).

3.2. Worm counts

At the first slaughter point (day 13) both diet ($P = 0.036$) and weaning status ($P < 0.001$) significantly affected total worm burden (Table 3). Ewes fed the low quality diet harboured higher numbers of *T. colubriformis* than those fed the high quality diet and lambing groups had higher numbers than dry ewes. By day 41, the effect of diet quality was lost, however, the effect of weaning status remained highly significant ($P < 0.001$). Suckled ewes harboured more *T. colubriformis* than their early-weaned and dry counterparts. There was no significant interaction between diet and weaning status at either kill time.

3.3. Circulating eosinophils

Ewes fed the high quality diet had lower ($P = 0.006$) circulating eosinophils during P1, but higher ($P = 0.056$) circulating eosinophils during P2. Blood eosinophils were unaffected by diet quality during P3. The effect of pregnancy status ($P < 0.001$) was evident from day –37 with pregnant ewes exhibiting lower eosinophil counts than dry ewes (Fig. 2). During P2, both lambing groups had significantly lower blood eosinophils than their dry counterparts ($P < 0.001$). From day 1 post-lambing, eosinophil counts of suckled ewes remained at low levels whereas those of early-weaned ewes began to rise. By day 6, counts of early-weaned ewes were significantly higher than suckled counts. There was no significant interaction between the effects of diet quality and weaning status during P2.

During P3, the effect of weaning status continued to have an effect on blood eosinophil concentrations ($P < 0.001$) with suckled ewes having significantly lower counts. Early-weaned ewes had mean eosinophil concentrations that exceeded the levels in dry ewes whereas the levels of infected suckled ewes remained low. During P3, early-weaned ewes had circulating eosinophil concentrations (raw) that, on average, were 3.8-fold that of the suckled ewe group.

Table 2

Mean faecal worm egg counts (backtransformed means) and 68% confidence intervals of Merino ewes fed either a high or low quality diet, that were either pregnant with single lambs or dry during period 1, then either early-weaned, suckled or dry during periods 2 and 3. Significance of treatment effects appear under the relevant experimental period.

	Period 1			Period 2					Period 3		
	–50*	–24*	–17*	–10*	–3*	4*	11*	18*	25*	32*	38*
H-P	0	0	1								
	0–0	1–3	1–2								
L-P	0	0	0								
	0–0	1–3	2–4								
H-D	0	2	1	1	0	0	0	0	1	0	0
	0–0	0–1	0–2	0–2	0–1	0–1	0–1	0–1	0–3	0–1	0–1
L-D	0	2	3	0	0	0	0	0	1	0	0
	0–0	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–3	0–1	0–1
H-EW				1	2	2	6	1	1	1	1
				0–1	1–4	1–4	3–12	0–4	0–5	0–4	0–4
L-EW				6	4	17	26	2	8	3	4
				3–10	2–8	9–31	14–46	0–6	3–20	1–9	2–10
H-S				3	3	3	8	4	5	24	100
				1–6	1–7	1–7	4–17	1–11	1–16	10–57	46–213
L-S				3	20	30	92	107	389	452	998
				2–6	11–37	16–56	49–173	45–251	148–1022	196–1040	469–2124
Preg	P=0.004										
Diet	n.s.			Diet	P=0.021				Diet	P=0.025	
P × D [#]	n.s.			Wean	P<0.001				Wean	P<0.001	
				DxW	n.s.				DxW	P=0.076	

Treatment group abbreviations: H = high quality diet, L = low quality diet, D = dry, P = pregnant, EW = early weaned, S = suckled.

* Days from the midpoint of lambing (day 0). The significance of effects “Preg”, “Diet” and “P × D” are for the period under which they appear.

[#] Pregnancy × Diet interaction.

3.4. Circulating neutrophils

Blood neutrophil concentration was unaffected by diet during all periods of the experiment. From day –37 (P1), pregnant ewes had higher blood concentrations of neutrophils ($P<0.001$) than dry ewes (Fig. 3). The spike in blood neutrophils occurring on day –7 in the dry treatment group was due to 4 ewes (2 from each of the high [$n=16$] and low [$n=15$] diets) exhibiting elevated levels. Weaning status significantly contributed to the variation in neutrophils ($P<0.001$) during P2. Neutrophils rose in the lambing groups then declined rapidly from day 0 and by

day 6, early-weaned ewes had counts significantly lower than suckled ewes. Weaning status of ewes continued to affect neutrophil levels ($P=0.042$) during P3 with suckled ewes having higher counts than early-weaned ewes ($P=0.039$).

3.5. Total Ab in plasma

Throughout all periods of the experiment, plasma total Ab was unaffected by diet quality. By day –24 (P1) pregnant ewes had significantly lower levels of total Ab in plasma than their dry counterparts (Fig. 4).

Table 3

Total *T. colubriformis* worm counts (backtransformed means), 68% confidence intervals and establishment rates of worms in Merino ewes on days 13 and 42 relative to the midpoint of lambing after being fed either a high or low quality diet and either dry, early-weaned or suckled.

Treatment group	Kill 1 – day 13			Kill 2 – day 41		
	Worm Count	68% CI (lower, upper)	Est. Rate	Worm Count	68% CI (lower, upper)	Est. Rate
High – dry	1	(0, 3)	0%	8	(3, 23)	1%
High – early-weaned	440	(166, 1165)	13%	8	(2, 23)	1%
High – suckled	1873	(645, 5440)	11%	4802	(1590, 14504)	21%
Low – dry	23	(9, 57)	2%	3	(1, 10)	1%
Low – early-weaned	3072	(1322, 7136)	19%	189	(67, 527)	7%
Low – suckled	2433	(988, 5991)	12%	11978	(3966, 36173)	46%
Wean <i>p</i>	<0.001			<0.001		
Diet <i>p</i>	0.036			ns		
Wean × Diet <i>p</i>	ns			ns		
C1: EW vs. suckled <i>p</i>	ns			<0.001		
C2: Lambd vs. dry <i>p</i>	<0.001			<0.001		

C1 refers to the statistical contrast of early weaned (EW) and suckled ewes. C2 refers to the contrast of ewes that lambed (i.e. EW and suckled) with those that remained dry. Est. Rate: Establishment rate was an estimate calculated from the total number of *T. colubriformis* worms present (raw counts) at each kill time expressed as a percentage of the total number of *T. colubriformis* larvae given over the previous 5 week period, under the assumption that each subset of ewes killed was representative of each other.

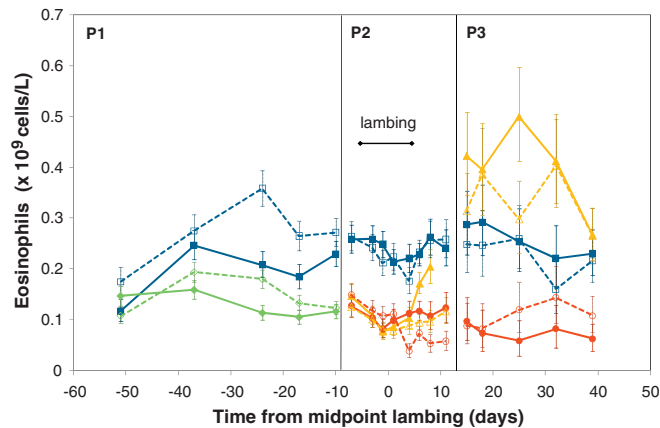


Fig. 2. Mean blood eosinophil concentration (backtransformed lsmeans $\pm$ 68% c.i.) of Merino ewes. During P1 ewes were either pregnant with single lambs (◆) or dry (■). During P2 and P3, ewes were either early-weaned (▲), suckled (●) or dry (■) during P2 and P3.

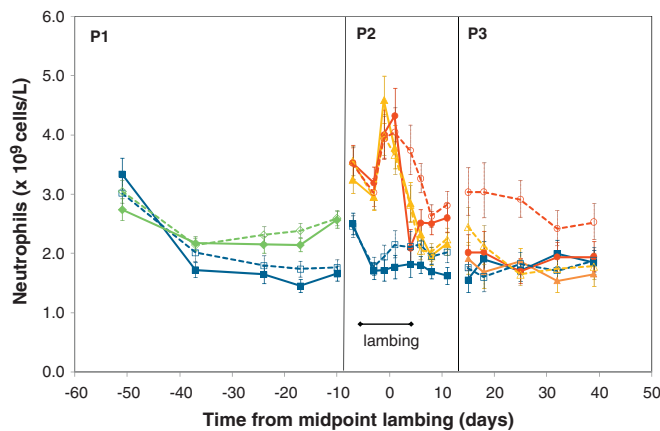


Fig. 3. Mean blood neutrophil concentration (backtransformed lsmeans $\pm$ 68% c.i.) of Merino ewes. During P1 ewes were either pregnant with single lambs (◆) or dry (■). During P2 and P3, ewes were either early-weaned (▲), suckled (●) or dry (■) during P2 and P3.

During P2, both lambing groups had significantly lower total Ab titres than dry ewes ($P < 0.001$) and by day 11, plasma titre of the early-weaned ewes had increased to become significantly greater than that measured in

suckled ewes. During P3, plasma total Ab in early-weaned ewes continued to rise, exceeding those in dry ewes, and maintaining levels significantly greater than suckled ewes ($P < 0.001$).

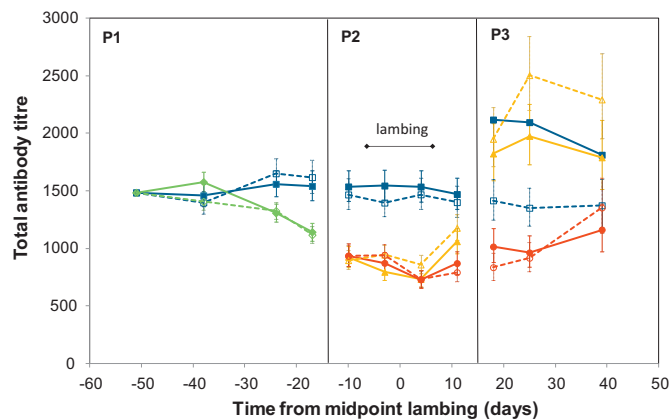


Fig. 4. Mean total antibody titre (backtransformed lsmeans $\pm$ 68% c.i.) of Merino ewes. During P1 ewes were either pregnant with single lambs (◆) or dry (■). During P2 and P3, ewes were either early-weaned (▲), suckled (●) or dry (■) during P2 and P3.

3.6. Tissue antibodies

The significance of the effects of diet and weaning status and of particular experimental contrasts on tissue antibody titre on days 13 and 41 is provided in Table 3. Ewes fed the high quality diet had higher titres of total Ab on days 13 and 41 (Fig. 5). Weaning status significantly affected total Ab, IgG₁, IgM and IgA titre on day 13 with suckled ewes having lower titres than their early-weaned counterparts. This effect was particularly noticeable for IgA, where the early-weaning of ewes resulted in a large increase, regardless of diet quality (Fig. 5). Weaning status remained as a significant contributor to the variation in tissue total Ab and IgG₁ at day 41 (Fig. 5). The significance of contrast C1 (Table 4) for these same isotypes indicated that suckled ewes had significantly lower titres than their early-weaned counterparts. IgG₂ titres were negligible (maximum back-transformed titre of 5.1) throughout the entire experimental period and were unaffected by diet, weaning status or any two-way interactions between these effects (Fig. 5).

3.7. Tissue mast cells, globule leukocytes and goblet cells

Numbers of MMC and GL present in small intestinal tissue were unaffected by diet at both kill times, however, number of GC were significantly higher in ewes fed the high quality diet on days 13 ($P < 0.001$) and 41 ($P < 0.001$). Mean (raw) number of GC at day 13 in ewes fed the high quality diet was 186 vs. 70 in ewes fed the low quality diet (a 2.7-fold difference). Similarly, at day 41, ewes fed the high quality diet had a mean of 146 vs. 51 in ewes fed the low quality diet (a 2.9-fold difference).

Weaning status was a significant contributor to the variation in numbers of tissue MMC, GL and GC at both kill times. On day 13, both lambing groups had significantly lower numbers of MMC ($P = 0.002$), GL ($P < 0.001$) and GC ($P = 0.013$) (Fig. 6). At the second kill (day 41), weaning status continued to affect all cell types with suckled ewes having lower numbers of MMC ($P < 0.001$), GL ($P = 0.001$) and GC ($P = 0.054$) than early-weaned ewes.

3.8. Ewe dry matter intake

Diet quality significantly affected DM intake of ewes during all periods of the experiment ($P < 0.001$) with ewes fed the high quality diet having a higher mean intake than those fed the lower quality diet (raw values of 1,577 vs. 946 g DM/d respectively) (Fig. 7). In terms of DM intake per kg body weight, ewes fed the high quality diet consumed, on average, 1.4-fold that consumed by ewes fed the low quality diet during P3 (31.9 vs. 22.9 g DM/kg body weight).

During P1, pregnancy resulted in ewes having significantly higher DM intake ($P < 0.001$) by day -31. During P2, DM intake was significantly affected by weaning status ($P < 0.001$), with suckled ewes consuming more feed on average than their early-weaned or dry counterparts. During P3, weaning status continued to be a significant contributor to the variation in DM intake of ewes ($P < 0.001$) with suckled ewes consuming more feed than both early-weaned and dry ewes. Within ewes fed the high quality diet, suckled ewes increased their DM intake throughout

the lactation period until it was, on average, 1.5-fold that of the early-weaned ewes (44.8 vs. 37.9 g DM/kg body weight, respectively). At the end of the experiment, suckled ewes on the high quality diet consumed 2.1 kg DM/d (raw value). Similarly, within ewes fed the low quality diet, suckled ewes had a higher DM intake compared to their early-weaned counterparts (raw values: 26.7 vs. 22.4 g DM/kg body weight, respectively) though the relative increase (1.2-fold) in DM intake was smaller.

3.9. Ewe maternal body weight

Ewes fed the high quality diet had higher maternal weights during all periods ($P < 0.001$) (Fig. 8). Weaning status did not significantly affect maternal weight during P2, however, during P3 the effect was significant ($P = 0.024$) with both lambing groups having lower maternal weight than their dry counterparts ($P = 0.020$). Within ewes fed the low quality diet, suckled ewes had significantly lower maternal weights than their dry counterparts on all days and significantly lower weights than early-weaned ewes on days 25, 32 and 38. No significant interaction between diet and weaning status existed during P2 or P3.

3.10. Ewe fat scores

Diet quality contributed to variation in FS of ewes during all periods of the experiment ($P = 0.008$, $P < 0.001$ and $P < 0.001$ for P1, P2 and P3 respectively) with ewes fed the high quality diet having higher scores (Fig. 9).

Fat scores were unaffected by pregnancy status during P1. During P2, weaning status significantly affected FS ($P < 0.001$) with both lambing groups having lower scores than their dry counterparts ($P < 0.001$). There was a significant interaction between weaning status and diet during P2 ($P = 0.028$) with ewes fed the low quality diet exhibiting a larger difference between lambing and dry ewes than was evident within the ewes on the high quality diet. During P3, suckled ewes had significantly lower FS than both their early-weaned and dry counterparts ($P = 0.002$).

3.11. Ewe fat and eye muscle depth

At day -16, both FD and EMD of pregnant ewes was less than that of dry ewes ($P = 0.004$ and $P = 0.001$ respectively) (Fig. 10). On day 9, following parturition and the early weaning of lambs, dry ewes continued to have significantly higher FD and EMD measurements than ewes that had lambed ($P < 0.001$).

Thirty-two days after the midpoint of lambing, suckled ewes had the lowest measurement of FD ($P = 0.022$) and EMD ($P = 0.005$), and the early-weaned and dry groups did not differ significantly. Between days -16 and 32 relative to the midpoint of lambing, FD and EMD of dry ewes remained fairly constant (within 0.3 mm and 0.1 mm respectively). Pregnant ewes that continued to suckle lambs lost 0.4 mm of FD and 1.9 mm of EMD over the experimental period whereas early-weaned ewes gained 0.3 mm of FD and 0.4 mm of EMD.

Ewes fed the high quality diet had significantly higher measurements of FD and EMD on days -16 ($P = 0.020$,

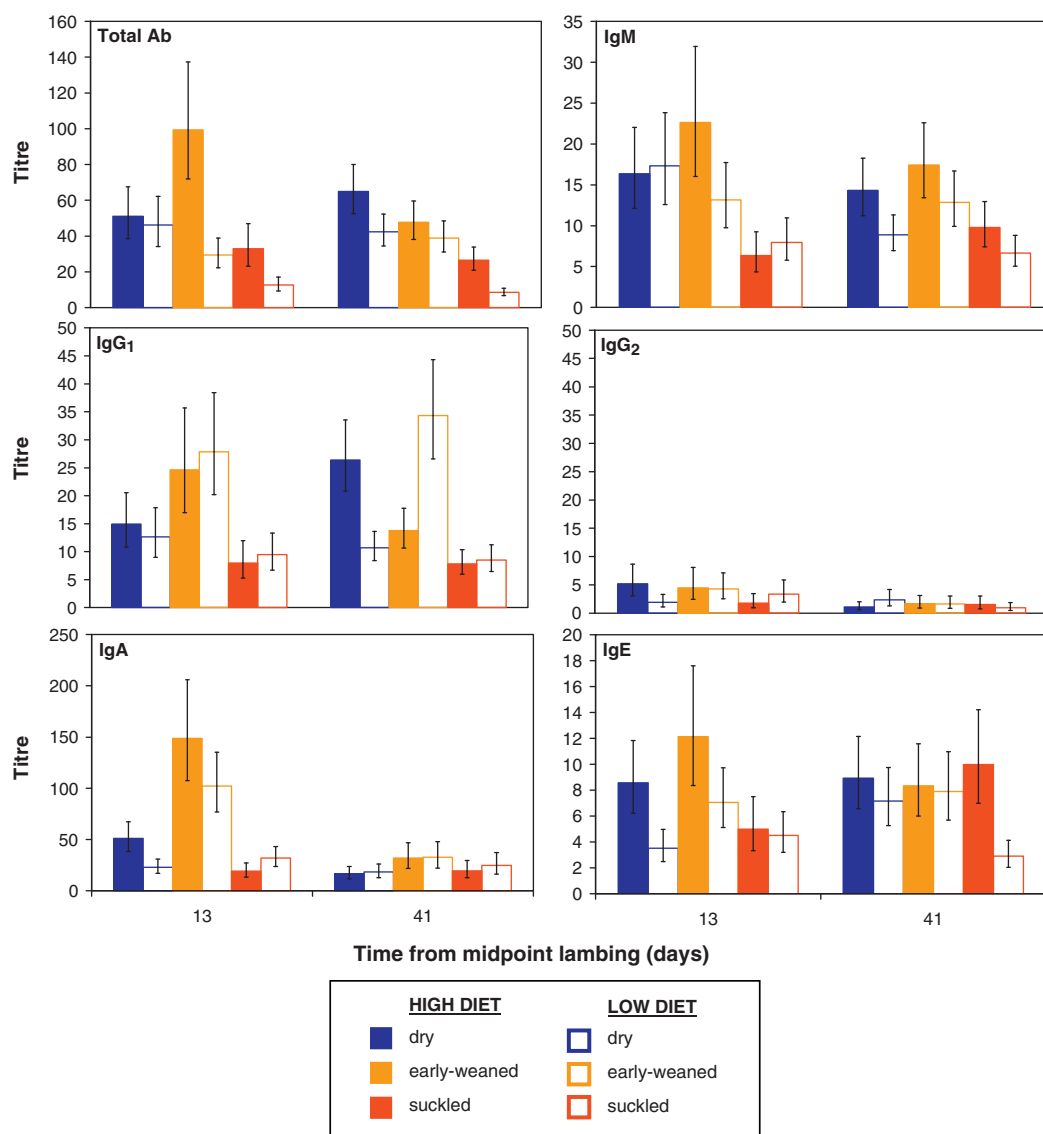


Fig. 5. Mean total antibody, IgM, IgG₁, IgG₂, IgA and IgE titres (backtransformed $\text{lsmeans} \pm 68\%$ c.i.) in small intestinal tissue of Merino ewes infected with *T. colubriformis* that were either fed the high (filled bars) or low quality diet (unfilled bars) and dry (■), early-weaned (■) or suckled (■). Note that y-axis scales differ between isotypes and that comparison between isotypes is not possible due to differences in binding capacities.

$P < 0.001$ respectively), 9 ($P < 0.001$ for both) and 32 ($P < 0.001$ for both) than ewes fed the low quality diet. Between days –16 and 32, the difference in FD and EMD between ewes fed the high and low quality diet increased from 0.4 mm to 1.0 mm (FD) and from 1.7 mm to 3.2 mm (EMD). The interaction between the effects of diet and weaning status was not significant during P2 or P3.

3.12. Lamb birth weight and growth rate and ewe milk production

Lambs born to ewes fed the high quality diet were significantly heavier at birth, on days 18, 32 and 38 than those born to ewes fed the low quality diet. Least square means (kg) $\pm$ s.e., and P values for these time points respectively were 4.6 ± 0.09 vs. 4.1 ± 0.09 ($P < 0.001$), 9.3 ± 0.48 vs.

7.0 ± 0.48 ($P = 0.006$), 12.3 ± 0.58 vs. 7.9 ± 0.58 ($P < 0.001$), and 13.3 ± 0.60 vs. 8.5 ± 0.60 ($P < 0.001$). Furthermore, growth rate between birth and day 38 was higher for lambs with dams on the high diet than lambs with dams on the low diet (373 vs. 224 g/d; $P < 0.001$). Average milk production of ewes, calculated from birth until day 38 was also significantly higher when fed the high quality compared to the low quality diet (2.2 vs. 1.2 kg/d, $P < 0.001$).

3.13. Leptin

Ewes fed the high quality diet had higher levels of blood leptin during P1, P2 and P3 ($P < 0.001$) (Fig. 11). Between days –37 and –7 (P1), pregnant ewes had significantly lower blood leptin than their dry counterparts. During P2, weaning status had a significant effect on blood leptin

Table 4

The significance of the main effects of weaning status and diet quality as well as particular contrasts (C) on days 13 and 41 relative to the midpoint of lambing for total Ab, IgG₁, IgG₂, IgM, IgA and IgE measured in small intestine tissue homogenate of Merino ewes infected with *T.colubriformis*.

	Total Ab	IgG ₁	IgG ₂	IgM	IgA	IgE
Kill 1—day 13 from midpoint of lambing						
Wean (W)	0.007	0.014	ns	0.018	<0.001	ns
Diet (D)	0.004	ns	ns	ns	ns	<i>0.082</i>
W × D	ns	ns	ns	ns	ns	ns
C1	0.004	0.004	ns	0.012	<0.001	<i>0.071</i>
C2	ns	ns	ns	ns	<i>0.071</i>	ns
C3	ns	ns	ns	ns	ns	ns
Kill 2—day 41 from midpoint of lambing						
Wean (W)	<0.001	0.002	ns	<i>0.085</i>	ns	ns
Diet (D)	0.003	ns	ns	<i>0.075</i>	ns	<i>0.068</i>
W × D	ns	0.003	ns	ns	ns	ns
C1	<0.001	<0.001	ns	0.027	ns	ns
C2	<0.001	ns	ns	ns	ns	ns
C3	0.050	ns	ns	ns	ns	ns

C1: early-weaned vs. suckled, C2: lambing groups (early-weaned and suckled) vs. dry group, C3: does infection status influence early-weaned ewes and suckled ewes equally? Italics indicates trend $P > 0.05$ but < 0.10 .

levels ($P < 0.001$) with early-weaned levels increasing rapidly following weaning to reach dry levels and suckled levels remaining low.

Differences due to weaning status continued to be evident into P3 ($P < 0.001$) with suckled ewes having the lowest levels of blood leptin and early-weaned and dry ewes having the highest levels. There was a significant interaction between weaning status and diet quality during P2 ($P = 0.002$) and P3 ($P = 0.004$). Following lambing, diet quality resulted in differences of between 0.9 and 1.3 ng/mL in both early-weaned and dry groups. In contrast, leptin levels of suckled ewes were unaffected by diet.

3.14. Associations among traits

Pearson correlation coefficients between all variables measured during P2 (since no data exists for post-mortem traits during P1) and P3 are shown in Table 5. Within the 132 correlations tested, 76 reached a level of significance of $P < 0.05$. However, after application of the Holm-Bonferroni correction for multiple comparisons, 21 had a target significance level of $P < 0.0004$, and another 10 were significant at $P < 0.001$, a level which might be considered as approaching significance, given the conservative nature of this test.

Worm egg count and total *T. colubriformis* WC were significantly and positively correlated during P3. Total WC was negatively correlated with the number of MMC found in small intestinal tissue. WEC was also positively associated with blood neutrophil level.

During P2, blood leptin concentration correlated negatively with blood neutrophil concentration and positively with numbers of GC in small intestinal tissue, FD and EMD. During P3, blood leptin level was positively and strongly correlated with both FD and EMD.

FD and EMD were strongly and positively correlated to each other during P2 and P3. Fat depth during P2 was correlated negatively with neutrophil concentration. Eye muscle depth was also negatively associated with blood neutrophil concentration. During P3, EMD was positively correlated to tissue GC numbers.

4. Discussion

4.1. Parasitology

The nutritional treatments imposed on the ewes during this experiment resulted in each reproductive group displaying a PPR with vastly different characteristics. Despite WEC remaining low (< 200 epg, raw value) throughout P1 and P2, initiation of the PPR was signalled in pregnant ewes fed the low quality diet by day -24 . Once lambing had occurred, WEC of suckled ewes rose more rapidly and remained greater than for dry and early-weaned ewes for the remainder of the experiment. There was no evidence that peak WEC of suckled ewes fed the low quality diet was captured within the experiment as WEC were continuing to rise. The peak of the PPR in this group may have occurred later than 6 weeks post-lambing. In contrast, ewes fed the high quality diet displayed a delayed and transient PPR of very small magnitude (< 400 epg, raw value). Only on days 32 and 38 post-lambing did suckled ewes fed the high quality diet have a significantly higher WEC than their dry and early-weaned counterparts. These results are in agreement with the finding (Donaldson et al., 1998; Kahn et al., 1999, 2003a; Houdijk et al., 2000; McAnulty et al., 2001) that increased diet quality (particularly dietary protein) is associated with a reduction in WEC and worm burden. Furthermore, the results reported here extend the understanding of the effect of nutrition on the PPR by using dry and early-weaned ewes as a comparison. That suckled ewes fed the high quality diet barely exhibited a PPR, making them similar to early-weaned and dry ewes, suggests that improved diet quality can minimise the immunosuppressive effects of suckling.

Total *T. colubriformis* worm counts in ewes that had lambed and were fed the high quality diet at the first kill point (day 13 post-lambing) had more worms than their dry counterparts (1,873 vs. 1 worm, respectively). These differences in WC occurred 19 days prior to the small but transient increase in WEC. This suggests that resistance in the lambing ewes had waned to an extent that allowed greater establishment of incoming larvae, but fecundity in

Table 5

Pearson's correlation coefficients (r) for the regression between parasite, endocrine, immune and production traits for period 2 (below the diagonal) and period 3 (above the diagonal).

		PERIOD 3											
PERIOD 2		WEC	WC	Lep	Neu	Eos	P Ab	T Ab	T MC	T GL	T GC	FD	EMD
	WEC		0.92	-0.35	0.31	-0.30	-0.16	-0.41	-0.37	-0.11	-0.30	-0.33	-0.43
	WC	0.34		-0.48	0.39	-0.38	0.21	-0.46	-0.50	-0.17	-0.37	-0.37	-0.46
	Lep	-0.35	-0.44		-0.19	0.28	0.36	0.43	0.38	0.01	0.39	0.82	0.69
	Neu	0.44	0.33	-0.50		-0.02	0.07	-0.28	-0.19	0.03	-0.28	-0.17	-0.38
	Eos	-0.20	-0.23	0.33	-0.38		0.44	0.32	0.28	0.26	0.22	0.14	0.21
	P Ab	-0.23	-0.22	0.21	-0.19	0.36		0.61	0.07	0.09	0.13	0.22	0.09
	T Ab	-0.29	-0.16	0.15	-0.06	0.32	0.72		0.14	-0.06	0.38	0.36	0.40
	T MC	-0.25	-0.63	0.51	-0.21	0.23	0.31	0.27		0.21	0.30	0.37	0.44
	T GL	-0.21	-0.26	0.30	-0.30	0.66	0.58	0.35	0.27		0.07	-0.08	-0.02
	T GC	-0.31	-0.40	0.59	-0.34	0.18	-0.01	0.17	0.32	0.09		0.44	0.55
	FD	-0.33	-0.32	0.82	-0.45	0.23	0.21	0.29	0.41	0.32	0.53		0.77
EMD	-0.24	-0.20	0.65	-0.49	0.38	0.16	0.33	0.21	0.27	0.50	0.71		

After applying the Holm-Bonferroni correction for 132 comparisons, the target level of significance of $P < 0.05$ becomes $P < 0.0004$. Correlations reaching significance of $P < 0.0004$ are in bold and italics, and $P < 0.001$ are in bold. Correlations with significance of $P > 0.001$ are in grey text. Abbreviations: WEC = worm egg count; WC = total *T. colubriformis* worm count; Lep = leptin; Neu = neutrophils; Eos = eosinophils; P Ab = plasma total antibody; T Ab = tissue total antibody; T MC = tissue mast cells; T GL = tissue globule leukocytes; T GC = tissue goblet cells; FD = fat depth; EMD = eye muscle depth.

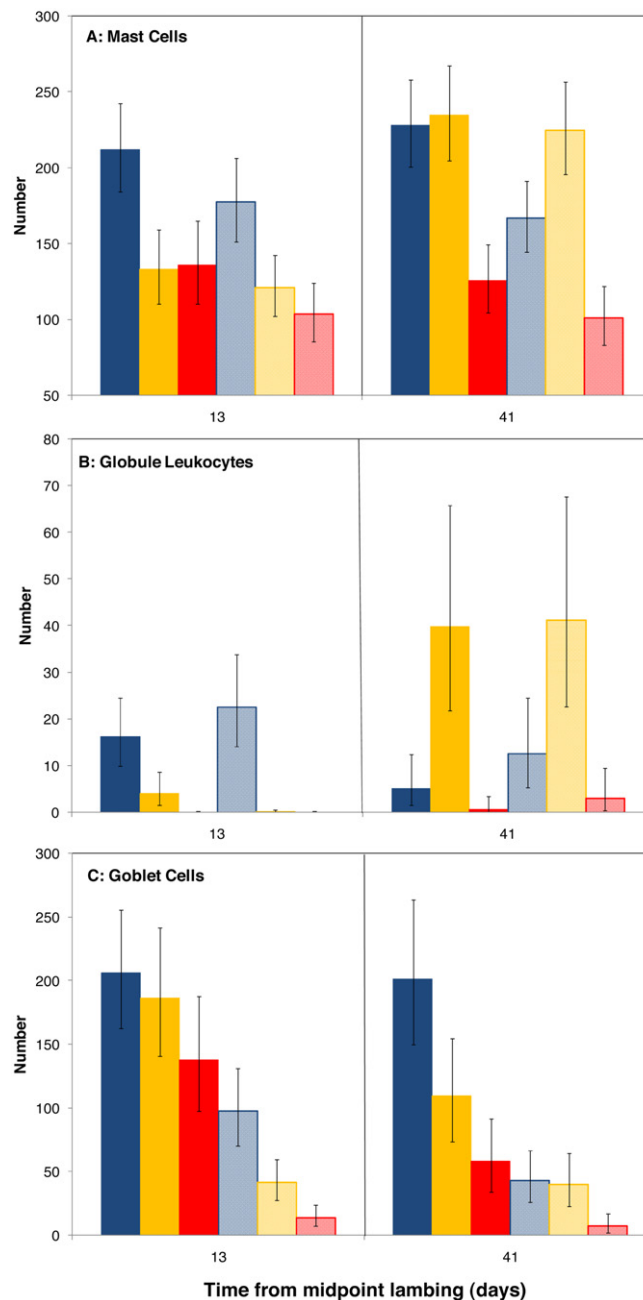


Fig. 6. Total number of mast cells (A), globule leukocytes (B) and goblet cells (C) (backtransformed $\bar{y}$ means $\pm$ 68% c.i.) counted in histological sections of infected Merino ewes on days 13 and 41 relative to the midpoint of lambing. Ewes were either dry (dark blue), early-weaned (yellow) or suckled (red), and fed either the high (darker) or low (lighter) quality diet.

these established worms remained low. It has been recognised that a manifestation of resistance in immune sheep is the ability to decrease fecundity of adult female worms (Bissett et al., 1996; Stear et al., 1999; Sutherland et al., 1999; Balic et al., 2000). In support of this, the correlation between WEC and WC for ewes fed the high quality diet during P2 was not significant ($r = 0.0326$, $P = 0.894$).

Similarly, within ewes fed the low quality diet, ewes that had lambed (either early-weaned or suckled) harboured more *T. colubriformis* worms at day 13 than dry ewes (2,433

vs. 23 worms, respectively). In contrast to ewes fed the high diet, the correlation between WEC and WC during P2 was positive and significant ($r = 0.4726$, $P = 0.026$) suggesting that fecundity was not greatly affected.

Dry ewes, regardless of the diet consumed, were able to maintain an adequate level of resistance to the *T. colubriformis* infection throughout the experiment with minimal larval establishment, and WEC not exceeding an average of 15 epg (raw count) and total worm count not exceeding an average of 657 (raw count). Similarly, leading up to day 13,

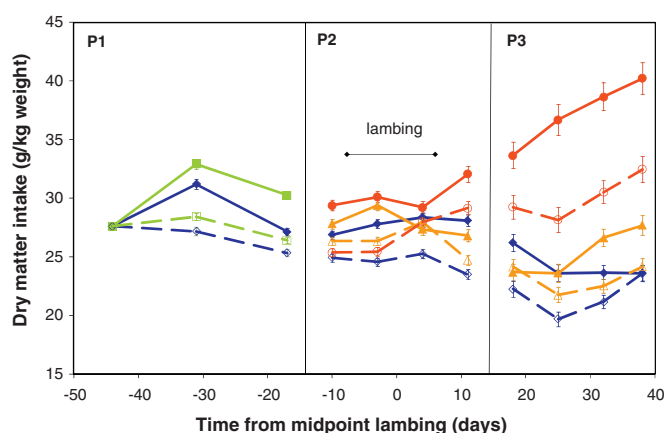


Fig. 7. Mean dry matter intake (backtransformed lsmeans $\pm$ 68% c.i.) of Merino ewes that were fed either the high (solid line and symbols) or low (dashed line and unfilled symbols) quality diet, and were either pregnant with single lambs (◆) or dry (■) during P1, then early-weaned (▲), suckled (●) or dry (■) during P2 and P3.

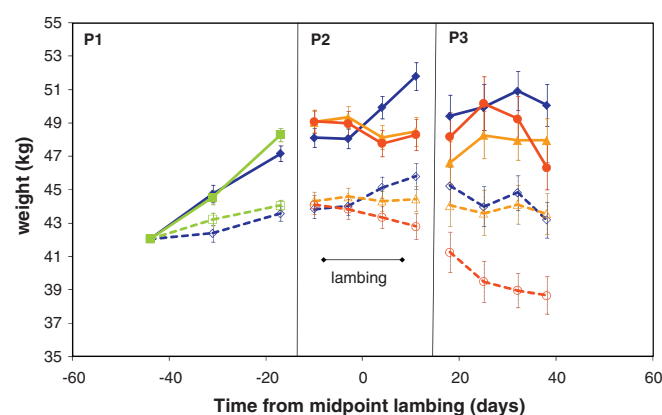


Fig. 8. Mean maternal body weight (backtransformed lsmeans $\pm$ 68% c.i.) of Merino ewes that were fed either the high (solid line and filled symbols) or low (dashed line and unfilled symbols) quality diet, and were either pregnant with single lambs (◆) or dry (■) during P1, then early-weaned (▲), suckled (●) or dry (■) during P2 and P3.

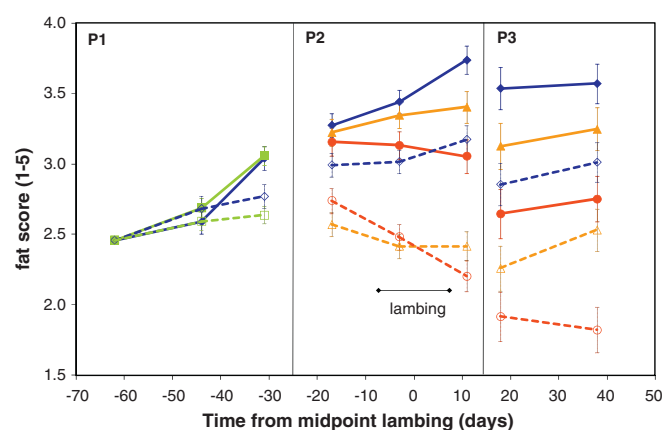


Fig. 9. Mean fat score (backtransformed lsmeans $\pm$ 68% c.i.) of Merino ewes that were fed either the high (solid line and filled symbols) or low (dashed line and unfilled symbols) quality diet, and were either pregnant with single lambs (◆) or dry (■) during P1, then early-weaned (▲), suckled (●) or dry (■) during P2 and P3.

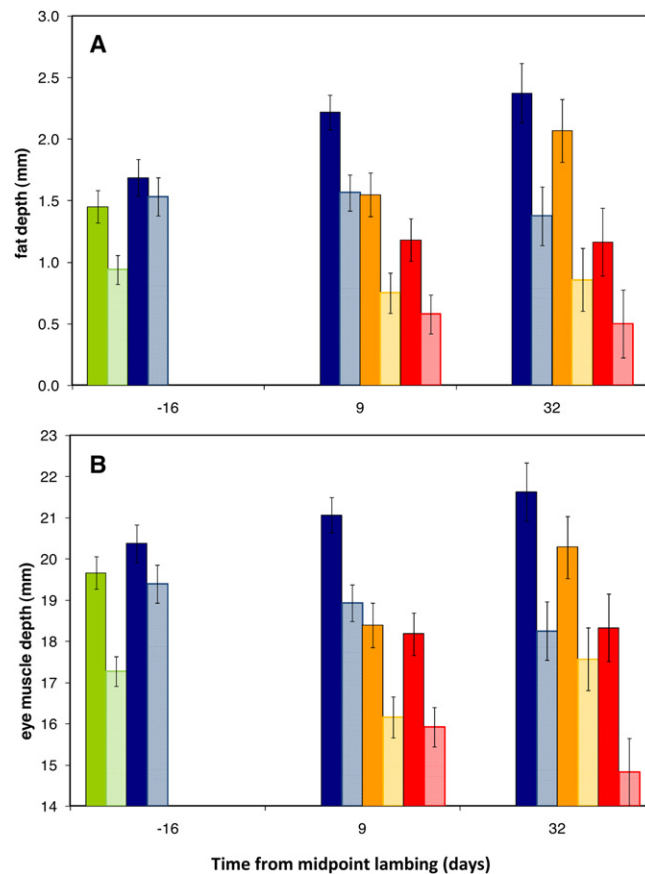


Fig. 10. Mean fat depth (A) and eye muscle depth (B) (backtransformed lsmeans $\pm$ 68% c.i.) of Merino ewes that were fed either the high (darker) or low (lighter) quality diet and were either pregnant with single lambs (green) or dry (blue) on day -16, then early-weaned (yellow), suckled (red) or dry (blue) on days 9 and 32.

establishment rates in all lambing ewes (either suckled or early-weaned) were generally low and did not differ greatly under the influence of diet. Resistance (as determined by the ability to limit larval establishment) in these classes of ewes, therefore, was not greatly influenced at this stage by

diet. By day 41 (kill 2), suckled ewes on the low quality diet allowed more than twice the number of larvae to establish than their counterparts on the high diet, and consequently their WC was more than 2-fold higher (14,831 vs. 6,576, raw counts, respectively) and they exhibited a marked PPR.

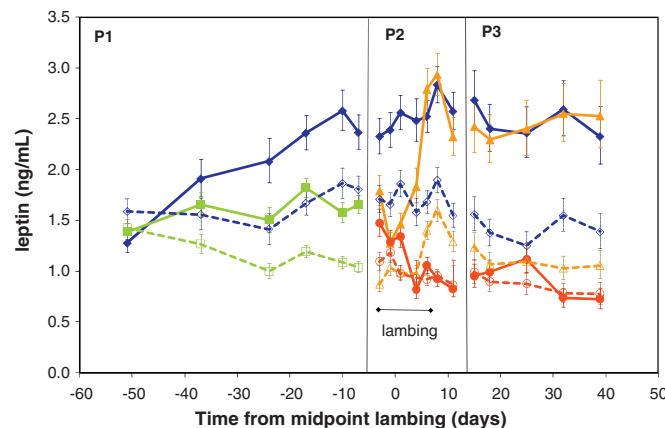


Fig. 11. Mean blood leptin concentration (backtransformed lsmeans $\pm$ 68% c.i.) of Merino ewes that were fed either the high (solid line and filled symbols) or low (dashed line and unfilled symbols) quality diet, and were either pregnant with single lambs (green diamonds) or dry (blue squares) during P1, then early-weaned (yellow triangles), suckled (red circles) or dry (blue squares) during P2 and P3.

Early-weaned ewes fed the high quality diet were able to reduce larval establishment (from 13 to just 1%) and worm burdens (by approximately 9-fold) between days 13 and 41. Even on the low quality diet, between days 13 and 41, early-weaning enabled ewes to reduce larval establishment by 2.7-fold (from 19 to 7%) and worm burdens by more than 2-fold. The recovery of resistance in early-weaned ewes shown here is consistent with the findings of Beasley et al. (2010b) and other reports in the literature (Salisbury and Arundel, 1970; O'Sullivan and Donald, 1970; Brunsdon and Vlassoff, 1971; Houdijk et al., 2006). However, the present experiment demonstrates that weaning leads to a rapid up-regulation of immunity even when animals are fed a low quality diet.

These results illustrate that diet quality had a large impact on the parasite status of ewes during the post partum period of infection with the low diet resulting in larger worm burdens and a marked PPR in suckled ewes. In general, ewes fed the high quality diet, even while suckling a lamb, were able to maintain a higher level of resistance preventing observation of a classical PPR. The large impact of diet on host resistance ensured that this experimental contrast would allow detection of mechanisms that underpin the role of nutrition in the PPR.

4.2. Immunity

The immune response to GIN is multifaceted consisting of range of interacting components and pathways that influence the dynamics of parasite population (Dobson and Barnes, 1995; Anthony et al., 2007) and manifestations of resistance (inhibition of the establishment of incoming infective larvae, reduced worm fecundity, altered sex ratios, and rejection of adult worms (Dineen, 1978). There is also an inbuilt redundancy, where different pathways can achieve the same end result (Anthony et al., 2007). Consequently, most observations for a specific mechanism involved in protection against GIN reported in the literature are associative rather than definitive. Furthermore, immunological reactivity can be modulated by a range of intrinsic and extrinsic factors (including, genetic constitution, age, sex, hormonal status influenced by stress, pregnancy, and lactation, nutritional status, and previous experience of infection) that act both quantitatively and qualitatively. For these reasons, descriptions of the course of immune events are experiment specific, but can be generally characterised as consisting of an initial antigen recognition phase (involving T and B lymphocytes and antibodies, followed by recruitment of effector cells by cytokines and their release of molecules that act on the parasite (Balic et al., 2000)). In the current experiment, we have chosen to focus on antibody responses that reflect antigen recognition and a range of circulating and local cell types having an effector function.

Immunity to GIN parasites in sheep is associated with elevated levels of all measured isotypes of antibody in serum (McClure et al., 1992) and also locally in gut tissue and mucus (McClure et al., 1992; Pfeffer et al., 1996). There have been variable responses of circulating antibody concentrations to feeding treatments (Van Houtert et al., 1995; Israfi et al., 1996; Sykes et al., 2007; Jones et al., 2011), but in

this instance and in line with the findings of Houdijk et al. (2005), diet did not significantly affect plasma total Ab titre during any phase of the experiment. Reproductive status, however, had a strong influence. Coinciding with the initiation of the PPR in ewes fed the low quality diet (day –24), total plasma Ab was decreased in pregnant ewes compared to dry ewes. This conforms to the results found previously by the current authors (Beasley et al., 2010b), and in general with others who describe impairments of specific antibody isotype responses during pregnancy (Ciupercescu, 1977; Reynolds and Griffin, 1990; Sykes et al., 2007). The reduction in plasma total Ab occurred in all pregnant ewes in this experiment, regardless of diet. However, only the ewes fed the low quality diet experienced a PPR at this stage, it could be concluded that systemic total Ab concentration was not a mechanism involved in the initiation of the PPR or regulated by host nutrition. Rather, it may be a reflection of the transfer of antibodies from the circulation during colostrumogenesis (Brandon et al., 1971; Cripps et al., 1976; Newby and Bourne, 1977; Hine et al., 2010). Overall, this supports the generally held view that systemic antibody levels provide a poor reflection of the local protective response to GIN parasites (Sinski et al., 1995; Else and Finkelman, 1998; Schallig, 2000). Despite this, the rapid recovery of systemic total Ab titre following early-weaning suggests a possible (intermediate) role in the modulation of the PPR in WEC.

A definitive role for eosinophils in resistance to GIN has yet to be fully described (Meeusen and Balic, 2000; Dombrowicz and Capron, 2001). The relationship between circulating eosinophils and resistance to *T. colubriformis* is complex as increasing numbers are associated with the acquisition of immunological responsiveness, but low numbers are found in both highly immune animals that have expelled their worms as well as highly susceptible animals (Windon, 1996). In the present experiment, the dynamics of the eosinophil response both within and between periods reflects this complexity and the superimposed modulating influences of physiological, hormonal, and nutritional factors. Circulating eosinophil response was the first component of the immune response to show down-regulation within pregnant ewes at day –37, 13 days prior to the initiation of the PPR in ewes fed the low quality diet. Diet quality, however, had inconsistent effects on eosinophil counts between reproductive periods. Although the PPR in WEC was initiated in pregnant ewes soon after a drop in circulating eosinophil level during P1, ewes fed the high quality diet had lower counts of blood eosinophils at this time. This suggests that reproductive physiology rather than dietary environment plays a greater role in the initiation of the PPR, but the rapid recovery of circulating eosinophil numbers following early-weaning, regardless of diet quality does provide evidence of at least an associative role in the PPRI.

Blood neutrophil concentrations were unaffected by diet throughout the experiment and therefore can be excluded as a potential link between nutrition and the PPR. Neutrophilia was evident in late pregnancy (from day –37) and in ewes suckling a lamb, with a brief spike at parturition. This generally conforms to periparturient neutrophil profiles reported elsewhere (Kehrli et al., 1989; Beasley et al., 2010b) and has been linked to increasing

levels of stress hormones, i.e. glucocorticoids, associated with parturition (Davis et al., 1991; Preisler et al., 2000; Madsen-Bouterse et al., 2006; Buckham Sporer et al., 2007), inflammation following soft tissue injury sustained during birth (Kelly, 1996), and bacterial assault associated with suckling of the lamb. Without contemporaneous profiles of blood cortisol in the present study, the link between glucocorticoids and neutrophilia cannot be confirmed or denied. However, under certain conditions, glucocorticoids can selectively down-regulate Th1 immunity (through decreased levels of IL-12, IFN- γ , and TNF- α), and neutrophil function (Davis et al., 1991; Goulding et al., 1998; Weber et al., 2004; Burton et al., 2005) and up-regulate Th2-mediated responses (through increased levels of IL-4, IL-10 and IL-13) (Elenkov et al., 1996; Ramirez, 1998; Elenkov and Chrousos, 1999; Miyaura and Iwata, 2002; Elenkov, 2004; Franchimont, 2004). Irrespective of the complex mechanisms involved, the inverse relationship between circulating eosinophil and neutrophil levels reported both here and by Beasley et al. (2010a) was noteworthy. Although only circumstantial, these results may suggest that a shift occurs from a Th2 to a Th1 type response during lactation rendering suckled ewes less capable of mounting an effective immune response against GIN. Early-weaned ewes might then revert back towards a Th2 response as a result of increasing glucocorticoid levels. Further experimentation is required to test this hypothesis.

Local effector responses, being free of the complexities associated with sequestration of antibodies for colostrogenesis and milk production, provide a more accurate assessment of the effects of diet and physiological status on the PPRI. The results presented here both support and add to the characterisation of local immunity impairment in lactating ewes reported by others. Houdijk et al. (2005), O'Sullivan and Donald (1973) and Huntley et al. (2004) reported a reduction in local MC, GL and eosinophil responses in the periparturient ewe. Similarly, this experiment also found that local immunity to *T. colubriformis* in lactating ewes was reduced as indicated by lower numbers of MC, GL, GC, and titres of total Ab, IgG₁, IgM and IgA in small intestinal tissue compared to their dry and early-weaned counterparts. A rapid recovery of local immune responses (total Ab, IgG₁, IgM, IgA and IgE, MC and GL in small intestinal tissue) occurred in early-weaned ewes demonstrating a requirement for lactational stimulus in maintenance of the PPRI. In contrast to the strong reproductive effects, the influence of improved nutrition was less pronounced, leading only to higher total Ab and number of GC measured in small intestinal tissue on days 13 and 41. There is no clear evidence for the influence of protein nutrition on local effector cells in the literature. Sykes et al. (2007) observed increased numbers of mucosal MC in infected periparturient ewes as a result of increased protein supply, but Houdijk et al. (2005) observed no significant effect of protein supply (0.8 vs. 1.3 times requirements) on MC, GL or eosinophil numbers in abomasal tissue of periparturient ewes infected with *T. circumcincta*. Houdijk et al. (2009) measured an increase in GL, but not in MC in response to metabolisable protein supply in *T. colubriformis*-infected lactating ewes. Protein supplementation has also been shown to enhance MC, GC and

eosinophil responses in lactating rats infected with *Nippostrongylus brasiliensis* (Jones et al., 2009, 2011). Despite physiological or reproductive status being the stronger contributor to the variation in local immunity in the current experiment, there is also evidence for a role of nutrition in affecting the magnitude of the PPR (during P2 and P3) via regulation of components of the local immune response to *T. colubriformis*.

4.3. Production

Based on diet quality and recorded dry matter intakes, the low diet was estimated to have provided MP and ME at 89, 100% during period 1; 58, 67% during period 2; and 33, 49% during period 3 of requirements (Kahn, 2003). The high diet was estimated to have provided MP, ME at 172, 132% during period 1, 123, 96% during period 2 and 81, 80% during period 3 of requirement. The MP supply from the high quality diet (104–138 g MP/day during periods 1–3) was similar to that used by Donaldson et al. (1998) to demonstrate the role of MP supply in regulating the resistance of periparturient ewes to GIN infection. In contrast, the MP supply from the low diet (50–60 g MP/day) was considerably less than the lowest levels typically used to investigate the role of diet to regulate host resistance (for example see, Donaldson et al., 1998; Houdijk et al., 2001a, b; Valderrabano et al., 2006).

The most notable productive effect of pregnancy and lactation was a loss of 45% of initial FD between days –16 and 32 in suckled ewes fed the low quality diet. A loss of 30–40% of the initial lipid store in 6 weeks is very common, rising to 80% of the body fat in underfed animals (Vernon et al., 1981; Chilliard et al., 2000). Protein catabolism also occurred in suckled ewes but the relative losses in EMD were substantially lower than observed for FD and net protein accretion after day 9 was supported by the high quality diet as evidenced by increasing EMD values. Fat loss during lactation is not always associated with a concomitant loss of protein (Cowan et al., 1979) for example, Masters and Mata (1996) found that ewes lost almost 50% of their fat reserves in the first 3 weeks of lactation, but only 16% of their body protein.

The data reported here allow comment on the apparent role of body reserves as the link between nutrition and the PPR, which in this experiment was manifest as rising WEC at day –24. Because the relative loss of EMD in suckled ewes between days –16 and 9 was similar for both diets (low quality 9%, high quality 8%) a greater emphasis on the importance of lipolysis (FD loss of: low quality 33%; high quality 14%) rather than protein catabolism in modulation of host resistance during the early stages of the PPR was apparent. Valderrabano and Uriarte (2003) and Valderrabano et al. (2006) suggested that body fat stored by ewes during pregnancy plays a role in the subsequent immune response to GIN around the time of parturition. Valderrabano and Uriarte (2003) found that ewes with greater backfat thickness (and presumably greater EMD, though data not reported) during early pregnancy had lower WEC (following mixed-species infection) until 3 weeks post-lambing while Valderrabano et al. (2006) reported a strong negative correlation between

average *Haemonchus contortus* WEC till lambing and body fat reserves at the time of infection (day 90 of pregnancy).

More quantitative studies conducted by other investigators (Donaldson et al., 1998; Houdijk et al., 2001a,b, 2003) have indicated that host resistance around parturition is more affected by endogenous (i.e. body protein mass) and dietary protein supply during late pregnancy and early lactation. For example, the classic work of Houdijk et al. (2001a) demonstrated that ewes fed to lose only body fat did not develop higher WEC (*T. circumcincta*) during lactation, whereas a loss of body protein and fat led to a loss of resistance. Our data supports the importance of changes in both fat and muscle to immunity of suckled ewes to *T. colubriformis*. For example, WC was negatively correlated with FD but not EMD during P2, the time at which FD was most sensitive to diet quality. In contrast, WC was negatively correlated with both FD and EMD during P3 when greater relative changes in EMD as a result of diet were observed. Nevertheless, there was no clear systematic relationship between changes in body composition with the parasite resistance status of ewes, although the physiological effects were pronounced. For example, dry ewes on the low quality diet had a similar nutritional status to suckled ewes on the high quality diet (as indicated by similar EMD on days –16, 9 and 32, and FD being only slightly greater for dry ewes), yet on days 13 and 41, WC of suckled ewes was 80 and 1600-fold respectively. This suggests that factors associated with lactation per se (independent of nutritional status) were involved in the periparturient relaxation of immunity in lactating ewes.

4.4. Leptin

The results presented here support the previously published positive relationship between plasma leptin and adiposity in the fed state in growing and lactating ruminants (Chilliard et al., 1998; Delavaud et al., 2000; Thomas et al., 2001), and also to acute variations in nutrient fluxes (Fantuzzi and Faggioni, 2000; Daniel et al., 2002; Delavaud et al., 2007; Beasley et al., 2010a) as seen here after lambing and early-weaning. The profile of plasma leptin during pregnancy, lactation and weaning observed in this experiment also conforms to previously published data (Block et al., 2001; Ehrhardt et al., 2001; Sorensen et al., 2002; Woodside et al., 2000).

The link between plasma leptin and the immune response, via regulation of cell-mediated immunity (Lord et al., 1998; Flier, 1998; Matarese, 2000; Ingvarsen and Boisclair, 2001; La Cava and Matarese, 2004; Matarese et al., 2005), provides a framework by which the nutritional status of the periparturient ewe could be translated into the PPR. Recent work, however, casts doubt on an immunoregulatory role for leptin in the aetiology and maintenance of the PPR. For example, Zaralis et al. (2009), observed that while protein supplementation of periparturient ewes infected with *T. circumcincta*, reduced the magnitude of the PPR it was unrelated to plasma leptin concentration. Plasma leptin levels reported here also appeared to be unrelated to observed changes in the immunity of periparturient ewes to *T. colubriformis*. Most striking was the large difference in plasma leptin during P3 for dry and

early-weaned ewes caused by diet, but not reflected in differences in worm count or WEC. In contrast, diet had no effect on the level of plasma leptin in suckled ewes during P3, yet WC and WEC differed by at least 2.5-fold between the high and low quality diets. These results suggest that lactation is a “leptin resistant” state and confirm that blood leptin is unlikely to play a causative role in the PPR.

4.5. Conclusion

The objective of this study was to investigate possible mechanisms that mediate the effects of nutrition on the resistance of periparturient ewes to GIN. Feeding ewes a low quality diet resulted in a PPR beginning by day –24 and increasing substantially throughout the lactation period in contrast to those fed a high quality diet which resulted in a delayed and transient PPR of very small magnitude. Results for various measures of immune competence have failed to identify a strong candidate for a mechanistic basis for nutrition to be the *initiating* factor in the PPR. Although there is ample reason to support nutritional initiatives in the quest to manage parasitism in breeding ewes in the future, some inconsistencies between body composition and apparent host resistance (by way of WEC and WC results) suggested that the nutritional influence was not necessarily always mediated through changes in body composition. Several factors that may mediate the effects of nutrition during the maintenance of the PPR were identified including circulating eosinophil concentration and total Ab titre and numbers of GC present in small intestinal tissue. Leptin did not fit the mould of a potential mediator between the nutritional environment and parasite status. In fact, the previous theories on leptin providing a link between nutrition and the periparturient relaxation of immunity to sheep worms have not been validated by the current data set since diet quality had no effect on blood leptin levels in suckled ewes yet resulted in drastically reduced WEC. It therefore might be concluded that some other factor associated with lactation, and perhaps not tightly mediated through changes in body composition, was contributing to the periparturient relaxation of immunity to *T. colubriformis*.

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