

1 Regulation of the resistance to nematode parasites of single and twin-bearing  
2 Merino ewes through nutrition and genetic selection

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11 **Abstract**

12 Periparturient Merino ewes obtained from lines of sheep that had been selected either  
13 for increased resistance to *Haemonchus contortus* (R) or at random (C) were supplemented,  
14 while grazing at pasture, with either nil or 250 g/day cottonseed meal for the 6 weeks prior to  
15 or the 6 weeks after the start of parturition. Ewes from both supplement groups had lower  
16 (mean 66% reduction) faecal egg counts (FEC) during the post partum period and this  
17 coincided with a period of maternal body weight loss. Factors which increased the rate of  
18 maternal body weight loss, such as pregnancy and lactation status, also increased FEC.  
19 Evidence is presented that the magnitude of the periparturient rise in FEC in grazing ewes will  
20 be greatest during periods of maternal weight loss and at these times supplementation to  
21 increase metabolisable protein supply will be most effective in increasing resistance to  
22 nematode parasites. The resistance of R ewes to nematode parasites was greater than that of  
23 C ewes throughout the experiment and was sufficiently low such that anthelmintic treatment  
24 in a commercial environment may not have been required. Irrespective of actual FEC, ewes  
25 from all treatment combinations exhibited a periparturient rise in FEC. Reduced FEC of R  
26 ewes resulted in reduced apparent pasture larval contamination after 18 weeks of continuous  
27 grazing but supplementation was ineffective in this regard. It is suggested that integrated  
28 parasite management programs for periparturient ewes should make use of both protein

1 supplementation and genetic selection to increase worm resistance and reduce dependency on  
2 anthelmintics for worm control.

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4 *Keywords:* Sheep-Nematoda, Protein supplementation, Nutrition, Genetic resistance,  
5 Metabolisable Protein, Periparturient

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## 7 **1. Introduction**

8         The development of anthelmintic resistance among nematode parasites and its high  
9 prevalence among Australian sheep farms (with increasing severity) has focused attention on  
10 the need to develop alternative worm control strategies for inclusion in integrated parasite  
11 management (IPM) programs (Love and Biddle, 2000). Two of the more promising and  
12 commercially feasible alternatives are genetic selection (Woolaston et al., 1990; Woolaston,  
13 1992) on the basis of low faecal egg count (FEC) shortly after weaning and improved protein  
14 nutrition (Bown et al., 1991, van Houtert et al., 1995; Donaldson et al., 1998). Of particular  
15 interest to the development of IPM programs is the targeting of these approaches to the  
16 livestock classes that contribute heavily to pasture larval contamination, namely the  
17 periparturient ewe and young sheep in the first 18 months of life.

18         During the periparturient period, sheep experience a temporary decline in worm  
19 resistance which leads to a periparturient rise (PPR) in faecal egg count. The PPR has a large  
20 influence on whole-farm worm control because it is a major source of pasture larval  
21 contamination (O'Sullivan and Donald, 1970; Lloyd, 1983). A number of causes, including  
22 endocrine-induced immunosuppression, for the PPR have been suggested (Barger, 1993) but  
23 the strongest evidence is that the magnitude of the PPR is largely controlled by both the  
24 worm-resistance status of the ewe (Woolaston, 1992) and the balance between the  
25 requirement and supply of metabolisable protein (MP) (Donaldson et al., 1998; Kahn et al.,  
26 1999; 2003; Houdijk et al., 2001a; 2001b).

1           The requirement for MP increases modestly during pregnancy but with the onset of  
2 lactation requirements increase rapidly in synchrony with milk yield. At peak lactation, MP  
3 requirement is about three fold greater (Freer et al., 1997) than in the nonreproductive state.  
4 Requirements for MP increase further with litter size and may be four-fold greater in twin-  
5 rearing ewes at peak lactation (Freer et al., 1997).

6           Coop and Kyriazakis (1999) proposed that the increased requirement for MP,  
7 associated with pregnancy and lactation, would take priority over the requirements necessary  
8 to support an effective immune response to worm infection and, when MP supply was not  
9 meeting requirements, predispose the periparturient ewe to the PPR. In support of this  
10 proposition, periparturient ewes infected with *Trichostrongylus colubriformis* and/or  
11 *Teladorsagia circumcincta* have been demonstrated to have significantly reduced FEC  
12 (Donaldson et al., 1998; Houdijk et al., 2001; Kahn et al., 2003) and worm counts (Donaldson  
13 et al., 1998) in response to an increased MP supply. The beneficial effects of an increased  
14 MP supply to the worm resistance of the periparturient ewe appears to be most pronounced in  
15 animals with the greatest MP requirement, namely twin-bearing and twin-rearing ewes  
16 (Donaldson et al., 1998; Houdijk et al., 2001).

17           Most of the experiments described earlier have been conducted with housed animals,  
18 receiving food at a constant availability and quality, and under these conditions the deficit of  
19 MP supply relative to requirement, termed the MP pressure (Kahn et al., 2003), will be  
20 greatest during lactation. The corollary of this experimental model is that the PPR will also  
21 be greatest during lactation. However, the MP pressure can be regulated by both an increased  
22 requirement or a reduced supply implying that maximal MP pressure and the PPR may not  
23 necessarily coincide with the period of greatest MP requirement.

24           In support of this, Kahn et al. (2003) demonstrated greatest benefits to the worm  
25 resistance of grazing Merino ewes, from increased MP supply during the prepartum rather  
26 than post partum period when MP requirements would have been greatest. Effects of

1 increased MP supply on worm resistance during the prepartum period occurred when ewes  
2 were experiencing maternal weight loss (-30 to -14 g/day). The absence of any benefits from  
3 increased MP supply to worm resistance during the post partum period occurred when ewes  
4 were experiencing maternal weight gain (80 to 100 g/day). These authors concluded that the  
5 period of maternal weight loss was the likely period of greatest MP pressure.

6         Recently, Kahn et al. (2003) reported that the effects of increased MP supply on the  
7 FEC of periparturient ewes were reduced considerably in grazing sheep derived from flocks  
8 selected for worm resistance. This is consistent with that reported for young sheep (Abbott et  
9 al., 1985). Kahn et al. (2003) suggested that the reduced immuno-responsiveness of worm-  
10 resistant sheep to MP supply may be a function of a higher existing priority for use of amino  
11 acids by the gut immune system and that gut immune responses to MP supply may not be  
12 enhanced beyond a certain level of mucosal immunity. Whether, these putative responses are  
13 compromised in worm-resistant animals with an even greater MP requirement (ie. twin-  
14 bearing ewes) or with a more restricted MP supply has yet to be determined.

15         This experiment was designed to extend the findings of Kahn et al. (2003) by testing  
16 the following hypotheses. Supplementation to increase MP supply of grazing periparturient  
17 ewes would (i) be most effective in reducing the extent of the PPR during periods of greatest  
18 MP pressure; and (ii) be most effective in reducing the extent of the PPR in ewes not selected  
19 for worm resistance. It was also hypothesised that both worm-resistant sheep and protein  
20 supplementation would result in lower levels of pasture larval contamination.

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## 22 **2. Materials and Methods**

### 23 *2.1. Experimental design*

24         A schematic representation of major experimental events is given in Fig. 1. Ewes  
25 were stratified within selection line on the basis of pregnancy status (ie. single or twin –  
26 bearing) and sire of progeny and then allocated at random to supplement group. The

1 experiment was a 2 x 3 factorial with 2 levels of genetic resistance and 3 supplement groups  
2 with 4 replicates for each treatment combination. Treatments were allocated at random to 24  
3 experimental plots (0.8 ha each with 9 ewes per plot) to constitute a randomised block design.  
4 Within each plot there were 6 single and 3 twin –bearing ewes which continuously grazed the  
5 same plots until lambs were on average 56 days of age. A fifth replicate of all experimental  
6 treatments was treated identically with the intention to use the animals as a source of  
7 replacement ewes to correct for lamb and ewe mortality during parturition. Replacements  
8 were made on the one occasion immediately after lambing ceased. The planned experimental  
9 period of 168 days was reduced to 127 days because of a number of factors of which blowfly  
10 strike, as a result of unseasonal prolonged wet weather, was the primary issue.

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## 12 2.2. *Animals and conditions*

13 Two hundred and sixteen mixed age (2-6 years of age) Merino ewes selected either for  
14 increased resistance (R) to *Haemonchus contortus* (n = 108) or at random (C) (n = 108)  
15 (Woolaston et al., 1990) were used in this experiment. Selection had been ongoing for the  
16 past 23 years. The ewes had been single sire mated (5 rams per selection line) over a 4 week  
17 period and were confirmed single (n = 144) or twin-bearing (n = 72) by ultrasonic scanning  
18 10 weeks after the start of mating.

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## 20 2.3. *Supplement group*

21 Animals were subjected to one of three supplementation strategies designed to provide  
22 on an as fed basis, 0 or 250 g/day cottonseed meal (CSM; 92% DM; 920 g OM/kg DM; 9 g  
23 phosphorus (P)/ kg DM; 313 g CP/kg DM; circa 50% rumen undegradable dietary protein)  
24 pellets fed for either 6 weeks prior to, or 6 weeks after, the start of lambing. Animals were  
25 fed three times per week on Monday, Wednesday and Friday mornings with the supplement  
26 being fed in a trough 3 m in length.

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2.4. Infection details

Preexisting infections were considered part of the experimental worm burden (Kahn et al., 2003). In addition, each ewe was infected with 3000 L<sub>3</sub> *Trichostrongylus colubriformis* and 1000 L<sub>3</sub> *H. contortus* larvae (both McMaster strain) on 3 occasions, those being 6, 5 and 3 days prior to allocation to experimental plots.

2.5. Sampling details

Timing of animal measurements is quoted with reference to the mid-point of lambing (day 0), 15 days after the start of lambing when c. 50% of lambs had been born (Fig. 1).

2.5.1. Tracer sheep

Prior to the start (days -85 to -72 inclusive), and after completion (days 93 to 106 inclusive) of the experiment, pasture larval numbers were assessed on the experimental plots according to the procedure described by Kahn et al. (2003). In brief, weaner rams (n = 48) from the *H. contortus* susceptible line were stratified on the basis of sire and 2 rams were allocated at random to each plot to act as tracer sheep. Tracers grazed in the plots, on both occasions, for a 2 week period before being removed and housed indoors. Four weeks later, faeces were collected from the tracers to determine FEC and cultured to determine the contribution of infective species to FEC.

2.5.2. Pasture sampling

Pasture was sampled from each plot on days -35 and 22 to assess herbage mass (kg DM/ha), and the percentage green and dead according to the procedure described by Kahn et al. (2003).

### 1 2.5.3. *Live weight and parasitology*

2 Ewes were moved to yards and weighed on days -71, -42, -26, 16 and 28. Lambs were  
3 weighed at birth and at weaning (17<sup>th</sup> January 2000) which was 69 days (day 125) after  
4 completion of the experiment. Faeces was collected from the rectum of ewes on the weighing  
5 days to estimate FEC using a modification of the McMaster method (lower level of detection  
6 100 epg). At each sampling, approximately equal amounts of faeces from each ewe within  
7 each plot were combined and cultured to facilitate microscopic identification of nematode  
8 larvae. Identification of nematode larvae was to genus level to determine genus contribution  
9 to FEC. Faecal egg count was not determined on lambs, because of the reduced duration of  
10 the experiment.

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### 12 2.5.4. *Supplement intake*

13 To estimate supplement intake of individual sheep, CSM pellets were sprayed with  
14 lithium chloride (LiCl; 0.83 mg Li (g pellet)<sup>-1</sup>) and fed on days -27 and 16. Following  
15 feeding, blood was sampled to determine plasma lithium concentration and from this  
16 supplement intake was predicted (Kahn, 1994).

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### 18 2.5.5. *Wool growth*

19 A 15 cm dyeband was placed on the midside position on the left side of ewes on days  
20 -71, -26 and 28 to determine wool growth. Wool growth rate, (g clean wool/day), washing  
21 yield and average fibre diameter were determined as described by Kahn et al. (2003).

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### 23 2.7. *Statistical analysis*

24 Six ewes were not pregnant and the data from these animals were omitted from  
25 analysis leaving a data set of 210 ewes. In addition, all data collected at day 56 was omitted

1 from analysis because a number of sheep were found to be suffering from flystrike which may  
2 have adversely affected the measured variables.

3 Data was analysed using a number of generalised linear models with the computer  
4 program SAS (SAS Institute Inc, 1990). The effects tested in the models used to analyse the  
5 experimental variables for the ewes were block, selection line, supplement group, parity  
6 (primi and multi-parous), age of animal (2-6 years), pregnancy status (single or twin),  
7 lactation status (0, 1 or 2 lambs) and first order interactions. Lactation status was included as  
8 an effect for data collected during the post partum period because of lamb mortality at  
9 parturition. Ewe bodyweight prior to the start of the trial was included as a covariate for  
10 subsequent weight measures. The significance of selection line, supplement group and the  
11 interaction between these effects was tested against block (within selection line x supplement  
12 group). All other effects were tested against the residual error term. In the analysis of  
13 experimental variables for the lambs the additional effects of lamb gender (male or female),  
14 birth type (single or twin; replaced pregnancy status) and rearing status (single or twin;  
15 replaced lactation status) and first order interactions were tested. Birth date was included as a  
16 covariate. The significance of selection line was tested against sire (within selection line).  
17 For all analyses, main effects and interactions which were not biologically or statistically  
18 significant were removed from the model.

19 The experimental unit for all analyses was defined as the experimental plot. Analysis  
20 of data was conducted in 3 discrete periods to avoid confounding of time with supplement  
21 group. The discrete periods were (1) prior to supplementation; (2) during the prepartum  
22 supplementation period; and (3) during the postpartum supplementation period. Repeated  
23 measures analysis was used in periods 2 and 3 when multiple samples were taken.

24 Faecal egg counts expressed as the number of eggs per gram of faeces (epg) were  
25 subjected to cube root transformation to normalise the data prior to analysis to determine

1 statistical significance and backtransformed means are presented in this paper. In all other  
2 cases least squares means (l.s.mean)  $\pm$  standard error (se) are used throughout the results.

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### 4 **3. Results**

5         There were insufficient replacement ewes to cover lamb and ewe mortality for all  
6 treatment combinations with twin-bearing and rearing ewes most affected. To ensure that all  
7 plots retained 9 ewes the balanced structure (ie. 6 single and 3 twin –bearing ewes per plot) of  
8 the experimental design was conceded. The final number of ewes within each treatment  
9 combination according to pregnancy and rearing status that was used for analysis of ewe  
10 variables is detailed in Table 1. To maintain 9 ewes per plot a small number (n=27) of single  
11 and twin-bearing ewes that lost their lamb/s, hence had a rearing status of zero, were  
12 maintained.

13

#### 14 *3.1. Tracer sheep*

15         Tracer rams developed patent nematode infections (backtransformed mean FEC 1844  
16 epg) indicating reasonable numbers of infective nematode larvae on the plots at the start of the  
17 experiment. Faecal egg count of tracer rams which grazed plots allocated to different  
18 treatment combinations did not differ indicating that there were no initial differences in larval  
19 numbers on pasture. However, *H. contortus* FEC was initially greatest (P<0.001) from tracers  
20 which grazed those plots where animals were allocated to receive supplementation.  
21 Backtransformed mean *H. contortus* FEC was 0 and 139 epg from tracers which grazed plots  
22 where animals were allocated to unsupplemented and supplemented groups respectively.  
23 *Teladorsagia* spp FEC was initially greatest (P<0.01) from tracers which grazed plots  
24 allocated to R sheep with backtransformed means being 938 and 309 epg for R and C lines  
25 respectively.

1 Faecal egg counts from tracer rams grazed after completion of the experiment were  
2 least ( $P < 0.05$ ) from those plots which had been grazed by R animals but were unaffected by  
3 supplementation and the interaction between these effects was not significant.  
4 Backtransformed mean FEC from tracer sheep which grazed plots previously grazed by R and  
5 C animals was 812 and 1618 epg respectively. Within total FEC, *H. contortus* and  
6 *Teladorsagia* spp FEC were lowest ( $P < 0.01$ ) from tracers which grazed plots which had been  
7 grazed by R ewes (Table 2). Although total FEC of tracers was unaffected by previous  
8 supplement group of ewes, *H. contortus* FEC was lowest ( $P < 0.01$ ) from tracers which grazed  
9 plots where ewes had been supplemented with CSM, with post partum supplementation being  
10 most effective (Table 2). Supplementation as a main effect did not influence  
11 *Trichostrongylus* spp or *Teladorsagia* spp FEC from tracer sheep but within supplement  
12 group, *Trichostrongylus* spp FEC was lower ( $P < 0.05$ ) from plots where animals had been fed  
13 prepartum. *Oesophagostomum* spp FEC from tracers was too low ( $< 20$  epg) to warrant  
14 meaningful interpretation of the effects tested.

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### 16 3.2. Herbage mass and species frequency

17 Green herbage mass did not differ between supplement group or selection line plots at  
18 any sampling period. Dead and total herbage mass were greatest ( $P < 0.01$ ) at day -35 in plots  
19 grazed by C sheep but this difference disappeared by day 22. The interaction between the  
20 effects of supplement group and selection line was not significant for measures of herbage  
21 mass. Least squares mean ( $\pm$  se) green herbage mass at days -35 and 22 was  $415 \pm 42.6$  kg  
22 and  $770 \pm 81.0$  kg DM/ha respectively. Least squares mean ( $\pm$  se) total herbage mass for  
23 plots grazed by R and C ewes was  $2083 \pm 191.8$  kg and  $2966 \pm 191.8$  kg DM/ha at day -35  
24 and  $2583 \pm 207.6$  kg and  $2663 \pm 207.6$  kg DM/ha at day 22. The most frequent pasture  
25 species were, in descending order, *Phalaris aquatica* (38%), *Holcus lanatus* (23%), *Lolium*  
26 *perenne* (21%), *Anthoxanthum odoratum* (6%) and *Bromus* spp (3%).

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3.3. Supplement intake of experimental ewes

Mean individual intake ( $\pm$  se) of supplement by ewes during the pre and post partum periods was estimated to be  $250 \pm 38.6$  and  $250 \pm 59.6$  g/day respectively. Including supplement intake as a covariate in the analysis of variance for FEC did not improve the fit of the model.

3.4. Parasitology of ewes

The species contribution to FEC is detailed in Table 3. Infections at day -42 were predominantly *H. contortus* (62%) and *Trichostrongylus* spp (24%) but by day 28 the contribution of *H. contortus* had decreased to 11% and that of *Trichostrongylus* spp increased to 54%.

Faecal egg counts of R ewes were significantly ( $P < 0.001$  at days -71, -42 and -26 and  $P < 0.02$  at days 16 and 28) lower than those of C ewes throughout the experiment (Fig. 2). The reduction in FEC of R ewes was consistent for *H. contortus* ( $P < 0.01$ ), *Trichostrongylus* spp ( $P < 0.02$ ) and *Teladorsagia* spp ( $P < 0.02$ ) FEC. Differences in *Oesophogostomum* spp FEC between R and C ewes were significant ( $P < 0.001$ ) only during the prepartum period.

Supplementation during the prepartum period did not affect FEC at days -42 and -26. The absence of an effect of supplementation during the prepartum period was consistent for *H. contortus*, *Trichostrongylus* spp, *Teladorsagia* spp and *Oesophogostomum* spp FEC. During the post partum period (ie. averaged over days 16 and 28) ewes from both supplementation groups had reduced FEC ( $P < 0.04$ ) (Fig. 2) relative to unsupplemented ewes. This reduction in FEC was largely accounted for by a reduced ( $P < 0.05$ ) *T. colubriformis* FEC. Backtransformed mean *T. colubriformis* FEC for the post partum period was 286 epg for unsupplemented and 97 epg for supplemented (mean of pre and post partum supplemented groups) ewes. The interaction between the effects of supplement group and selection line for

1 FEC was not statistically significant.

2 Faecal egg counts of single-bearing ewes were significantly ( $P<0.01$ ) lower than that  
3 of twin-bearing ewes during both the pre and post partum periods. This effect was consistent  
4 across the four dominant nematode species. Backtransformed mean FEC for single and twin-  
5 bearing ewes were very low over the prepartum period and were 8 and 27 epg respectively.  
6 During the post partum period, backtransformed mean FEC was 127 and 377 epg for single  
7 and twin-bearing ewes respectively. Lactation status and the interactions between the effects  
8 of pregnancy or lactation status with selection line and supplement group for FEC were not  
9 statistically significant.

10 Faecal egg counts of multiparous ewes tended ( $P<0.06$ ) to be lower than that of  
11 primiparous ewes during the prepartum period but FEC of both groups were very low and did  
12 not exceed 30 epg. This effect was significant for all nematode species ( $P<0.05$ ) with the  
13 exception of *H. contortus* FEC which did not differ significantly between multi- and  
14 primiparous ewes. Faecal egg counts during the post partum period were unaffected by  
15 parity. The interaction between the effects of parity with selection line and supplement group  
16 for FEC were not statistically significant.

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### 18 3.5. Body weight of ewes

19 Body weight at day -72 (preexperimental) did not differ among supplement groups  
20 and selection lines and the interaction between these effects was not statistically significant.  
21 Differences in body weight due to pregnancy status and parity were significant (both  $P<0.01$ )  
22 at day -72 at which time the least squares mean ( $\pm$  se) body weights were  $41.2 \pm 0.42$  and  $43.8$   
23  $\pm 0.63$  kg for single and twin-bearing ewes respectively and  $40.6 \pm 0.69$  kg and  $44.4 \pm 0.38$   
24 for primi- and multi-parous ewes respectively. Body weight at day -72 was a significant  
25 covariate ( $P<0.01$ ) for all subsequent measures of body weight.

26 Supplementation during the prepartum period increased ( $P<0.01$ ) body weights of

1 ewes at days -42 and -26 (Fig. 3). However, effects of supplementation during the prepartum  
2 period were not consistent between the selection lines with supplementation resulting in  
3 increased ( $P<0.05$ ) body weights of C but not R ewes. Least squares mean ( $\pm$  se) body  
4 weights, averaged over the prepartum period, for ewes that were either unsupplemented or  
5 supplemented prepartum were  $45.9^a \pm 0.29$  and  $46.5^a \pm 0.41$  respectively for R ewes and  $46.3^a$   
6  $\pm 0.29$  and  $48.3^b \pm 0.40$  kg respectively for C ewes. Supplementation did not effect body  
7 weight at day 16 but at day 28, body weights of ewes from both supplement groups were  
8 greater ( $P<0.05$ ) than unsupplemented ewes.

9       Body weights of C ewes were greater ( $P<0.05$ ) when averaged over the prepartum  
10 period but no differences existed during the post partum period. Least squares mean ( $\pm$  se)  
11 body weights for R and C ewes during the prepartum period were  $46.1 \pm 0.24$  and  $46.9 \pm 0.24$   
12 kg respectively. There were no differences in body weight between ewes of different parity  
13 and pregnancy status during the prepartum period. During the post partum period, ewes that  
14 had been single-bearing had greater ( $P<0.01$ ) body weights than those that had been twin-  
15 bearing but body weight was not significantly affected by lactation status. Least squares  
16 means ( $\pm$  se) for single and twin-bearing ewes were  $41.3 \pm 0.69$  and  $38.2 \pm 0.69$  kg at day 16  
17 respectively and  $40.2 \pm 0.72$  and  $36.5 \pm 0.72$  kg at day 28 respectively.

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### 19 *3.6. Wool growth – ewes*

20       Clean wool growth (g/day) of C ewes was greater ( $P<0.01$ ) than that of R ewes  
21 between days -72 to -26 (Period 1) but there were no differences between the lines between  
22 days -25 to 28 (Period 2) (Table 4). Mean fibre diameter of wool grown during the  
23 experiment did not differ between the selection lines but was numerically greater for C ewes.  
24 Clean fleece weight (annual wool growth; kg  $\pm$  se) of C ewes ( $2.5 \pm 0.05$  kg) was greater  
25 ( $P<0.03$ ) than that of R ewes ( $2.3 \pm 0.05$  kg) but there was no difference in mean fibre  
26 diameter.

1 Clean fleece weight was unaffected by supplementation during the experimental  
2 period and was subsequently used as a significant ( $P<0.001$ ) covariate to test whether  
3 supplementation affected the rate of clean wool growth. Supplementation did not increase  
4 clean wool growth rate during the pre or post partum periods. The interaction between the  
5 effects of supplement group and selection line was not significant for clean wool growth and  
6 fibre diameter

7 Single-bearing ewes grew 15% more ( $P<0.01$ ) wool than twin-bearing ewes between  
8 the days -72 to -26 and single lactating ewes grew 32% more ( $P<0.01$ ) wool than twin-  
9 lactating ewes between the days -25 to 28 (Table 4). Mean fibre diameter was not  
10 significantly affected by pregnancy status but single-lactating ewes had a greater ( $P<0.05$ )  
11 fibre diameter between days -25 to 28.

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### 13 *3.7. Birth weight and growth of lambs*

14 Birth date was a significant covariate for birth weight ( $P<0.05$ ), body weight of lambs  
15 at day 125 ( $P<0.01$ ) and for body weight gain from birth to day 125 ( $P<0.01$ ). The use of  
16 birth date as a covariate indicated that birth weight increased by  $15 \pm 6.4$  g/day during the 4  
17 week lambing period but by day 125, lambs were an extra  $103 \pm 40.0$  g heavier for every  
18 extra day of age.

19 Supplementation increased ( $P<0.05$ ) birth weight and lambs born to C ewes were  
20 heavier ( $P<0.05$ ) than those born to R ewes. Birth weight of single-born lambs and of lambs  
21 born to multiparous ewes was greater ( $P<0.001$ ) than that of twin-born lambs and lambs born  
22 to primiparous ewes respectively (Table 5). The effect of gender and interactions between the  
23 effects of selection line and supplement group were not significant for birth weight.

24 Body weight at day 125 and body weight gain from birth to day 125 were unaffected  
25 by supplement group, selection line and dam parity. However, R lambs, although being 0.3  
26 kg lighter at birth were 0.2 kg heavier by day 125. Body weight at day 125 and body weight

1 gain of single-born lambs and lambs reared as a single were greater ( $P<0.001$ ) than that of  
2 twin-born lambs and lambs reared as a twin. Male lambs were heavier than female lambs  
3 ( $P<0.05$ ) by day 125 (Table 5). The interaction between the effects of selection line and  
4 supplement group were not significant for body weight at day 125 or body weight gain.

5

#### 6 **4. Discussion**

7

8 Green herbage mass and the initial level of natural infection from pasture did not  
9 differ among experimental treatments although there were some initial differences for FEC of  
10 individual nematode species. These results indicate strongly that the parasitological and  
11 production effects observed were largely attributable to experimental treatments.

12 The results from this experiment support the first hypothesis that supplementation to  
13 increase the supply of MP is most effective in reducing the extent of the PPR during periods  
14 of greatest MP pressure. Supplementation did not reduce FEC during the prepartum period  
15 but post partum FEC of ewes which received either pre or post partum supplementation was  
16 reduced by 66% (526 epg for unsupplemented and 176 epg for mean of supplemented  
17 groups).

18 The reduction in post partum FEC as a result of supplementation coincided with the  
19 period of greatest body weight loss and presumably greatest MP pressure. Body weight  
20 change between days -26 to 28 (ie. largely the post partum period) for ewes (mean for ewes  
21 suckling either a single or twin lamb) was -168 g/day for unsupplemented, -194 g/day for  
22 prepartum supplemented and -134 g/day for post partum supplemented ewes. However, at  
23 day -26 (day 124 of gestation) ewes were carrying a conceptus and the estimated conceptus  
24 weight at this time was 3.7 kg for singles and 6.5 kg for twins (Black, 1983; Arthur et al.,  
25 1989). Deduction of the estimated conceptus weight indicated that, during the post partum  
26 period, ewes were losing maternal weight at the rate of -73 g/day for unsupplemented, -100

1 g/day for prepartum supplemented and -39 g/day for post partum supplemented. During the  
2 prepartum period, the estimated maternal weight gain after allowance for conceptus growth of  
3 50 g/day for singles and 89 g/day for twins (Black, 1983; Arthur et al., 1989) was 10 g/day  
4 and 49 g/day for unsupplemented and supplemented ewes respectively.

5 That the worm resistance of ewes was most responsive to supplementation during a  
6 period of maternal weight loss confirms the findings of Kahn et al. (2003). Those authors  
7 also found that supplementation to increase the MP supply of grazing Merino ewes was most  
8 effective during the period when ewes were experiencing maternal weight loss. In that study  
9 however, maternal weight loss occurred during the pre but not the post partum period.

10 Susceptibility of periparturient ewes to *T. circumcincta* infection is also increased  
11 during periods of ewe body weight loss (McAnulty et al., 2001) and it is apparent that a loss  
12 of body protein but not body fat predisposes periparturient ewes to the PPR during subsequent  
13 periods of high MP pressure (Donaldson et al., 2001; Houdijk et al., 2001a). The apparent  
14 importance of the size of the body protein pool in providing a labile source of amino acids and  
15 thereby minimising the PPR (Houdijk et al., 2001a) may also account, in the present study, for  
16 the reduced post partum FEC of ewes that were supplemented during the prepartum period.  
17 Prepartum supplementation increased maternal body growth rates and presumably protein  
18 mass which may have provided a larger pool of amino acids from which the gut immune  
19 system could have subsequently benefited from during the post partum period.

20 These results suggest that, during periods of lower MP pressure, increased MP supply  
21 will provide little benefit to resistance to nematode parasites but will increase maternal body  
22 mass and conceptus weights. In contrast, during periods of greater MP pressure, increased  
23 MP supply will benefit greatly the resistance to nematode parasites and will also be used to  
24 reduce losses of maternal body mass. While these findings support the central role of MP  
25 pressure in determining the magnitude of the PPR they do not provide an indication of the

1 strength with which MP is prioritised between the requirements of pregnancy and lactation  
2 and immunity to nematode parasites (Coop and Kyriazakis, 1999).

3         Increasing litter size also provides an opportunity to examine the partitioning of MP  
4 between immunity to nematode parasites and pregnancy and lactation. During the prepartum  
5 period, estimated maternal weight gain was positive for both single (39 g/day) and twin-  
6 bearing (7 g/day) ewes and during this period FEC was higher in twin-bearing ewes, but  
7 absolute values of FEC were low. During the post partum period, estimated maternal weight  
8 gain was negative for both single (-59 g/day) and twin-lactating (-82 g/day) ewes and FEC  
9 was substantially higher in twin-lactating ewes. That twin-lactating ewes experienced greater  
10 maternal weight loss and a greater PPR than single-lactating ewes indicates that the  
11 maintenance of maternal body mass and resistance to nematode parasites were both  
12 compromised but the existence of relative MP partitioning priorities were not obvious.

13         Support for hypothesis two, that supplementation to increase MP supply would be  
14 most effective in ewes not selected for worm resistance, was not apparent from the results of  
15 this study. The interaction between the effects of selection line and supplement group was not  
16 statistically significant for FEC and there was little indication that supplementation had a  
17 greater proportional benefit to the worm resistance of C ewes. However, the absolute  
18 reduction in post partum FEC as a result of CSM supplementation was considerably greater in  
19 C ewes. Averaged over the post partum period CSM supplementation reduced FEC of C  
20 ewes from 852 epg to 269 epg (a 68% reduction) and of R ewes from 199 epg to 83 epg (a  
21 58% reduction).

22         These results contrast with those reported by Kahn et al. (2003) who postulated that  
23 the lesser effect of CSM supplementation on reducing the FEC of ewes selected for worm  
24 resistance was a function of a greater priority by the gut immune system for amino acids. A  
25 change in protein metabolism as a consequence of selection for increased worm resistance  
26 appears an equally valid conclusion from the present study. Clean fleece weight and pre and

1 post partum wool growth rates of R ewes were consistently about 9% lower than that of C  
2 ewes and lamb birth weights of R ewes were 8% lower. One possibility to account for the  
3 apparent contradiction with that reported by Kahn et al. (2003) is that the MP pressure in the  
4 present study was considerably greater as indicated from estimated maternal weight loss. It is  
5 possible that the MP pressure was sufficiently great so that the gut immune response of R  
6 animals was compromised by an insufficient supply of MP.

7 In support of earlier publications (Woolaston et al., 1992; Kahn et al., 2003) FEC of R  
8 ewes were significantly less than those from C ewes throughout the periparturient period.  
9 Irrespective of the greater worm resistance, R ewes still exhibited a PPR (Woolaston et al.,  
10 1992). Indeed the FEC from ewes in all treatment groups exhibited a rise with the increase in  
11 FEC occurring earliest in unsupplemented C ewes. Examination of the literature indicates the  
12 persistence of the PPR despite differences in MP supply (Donaldson et al., 2001; Houdijk et  
13 al., 2001a) and apparent protein mass (Houdijk et al., 2001a). Taken together, it seems  
14 plausible to suggest that the PPR *per se* may be independent of MP supply but that its  
15 magnitude is highly regulated by dietary and endogenous MP supply.

16 The severity of nematode infection is a function of both host resistance and larval  
17 intake (Steel 1978). Nematode parasite control strategies that reduce larval numbers on  
18 pasture, through sustained lower FEC, will accrue benefits to parasite control through reduced  
19 larval intake. Continuous grazing of plots with R but not C ewes for an 18 week period  
20 subsequently resulted in a lower FEC of tracer rams indicating a reduction in pasture larval  
21 counts; with lowest FEC for *H. contortus* and *Teladorsagia* spp FEC. Supplementation was  
22 considerably less effective in lowering the FEC of tracer rams. Such effects are consistent  
23 with the impact of selection line and supplement group on mean experimental FEC. Ewes  
24 from the R flock had a mean experimental FEC 77% lower than C ewes while  
25 supplementation reduced mean (mean of R and C ewes) experimental FEC by 25%. The

1 benefits to a lower pasture larval count from worm resistant animals will increase further the  
2 differences in FEC between animals selected or not for worm resistance.

3 In conclusion, supplementation to increase the MP supply to grazing periparturient  
4 ewes is likely to be most effective in increasing resistance to nematode parasites during  
5 periods of maternal weight loss, particularly in ewes who may have had to mobilise body  
6 protein during pregnancy. In the context of the Australian sheep grazing industry this is a  
7 common scenario. The worm resistance of R ewes, after a 23 year period of selection, was  
8 such that anthelmintic treatment would have been unwarranted. The greater worm resistance  
9 of R ewes impacted on the epidemiology of infection by apparently lowering pasture larval  
10 contamination. Despite having lower levels of worm infection, R ewes did not exhibit any  
11 production benefits presenting the possibility that the partitioning of amino acids has been  
12 changed as a consequence of selection. Protein supplementation and genetic selection are two  
13 approaches that may make useful contributions to IPM programs for the periparturient ewe.

14

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22

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1 Table 1

2 Number of ewes within each treatment combination according to pregnancy and rearing  
3 status.

Selection line	Supplement group	Pregnancy status							TOTAL
		single			twin				
		Rearing status			Rearing status				
0	1	Subtotal	0	1	2	Subtotal			
Resistant	Nil fed	4	23	27	1	2	6	9	36
	Prepartum	3	19	22	0	5	7	12	34
	Post partum	5	21	26	1	3	6	10	36
	TOTAL								106
Control	Nil fed	6	16	22	2	5	6	13	35
	Prepartum	2	22	24	2	4	5	11	35
	Post partum	1	22	23	0	6	5	11	34
	TOTAL								104

4

5

6

- 1 Table 2
- 2 Backtransformed mean faecal egg count (FEC) and species contribution to FEC from tracer
- 3 rams grazed in experimental plots for 2 weeks after completion of the experiment.

	FEC (epg)	<i>Haemonchus contortus</i> (epg)	<i>Trichostrongylus</i> spp (epg)	<i>Teladorsagia</i> spp (epg)
<i>Selection line</i>				
Resistant	812 <sup>a</sup>	289 <sup>a</sup>	363	105 <sup>a</sup>
Control	1618 <sup>b</sup>	932 <sup>b</sup>	278	304 <sup>b</sup>
P value	0.04	0.003	NS	0.004
<i>Supplement</i>				
Nil fed	1318	826 <sup>a</sup>	308	152
Pre partum	1344	787 <sup>a</sup>	218	237
Post partum	885	211 <sup>b</sup>	462	180
P value	NS	0.01	NS	NS

- 4 Means within column and main effect with different superscripts differ significantly with P
- 5 value shown in table. NS, not statistically significant.
- 6 Note: The sum of individual species epg will not equate to the total FEC because the species
- 7 contribution to FEC was calculated prior to transformation of total FEC.

- 1 Table 3
- 2 Overall mean species ( $\pm$  se) contribution to faecal egg count of ewes determined from culture
- 3 of faeces pooled within plots.

Day	Nematode species (%)			
	<i>Haemonchus contortus</i>	<i>Trichostrongylus</i> spp	<i>Teladorsagia</i> spp	<i>Oesophogostomum</i> spp
-42	62 $\pm$ 6.8	24 $\pm$ 7.1	6 $\pm$ 1.8	8 $\pm$ 5.0
-26	29 $\pm$ 5.4	33 $\pm$ 5.1	17 $\pm$ 3.5	21 $\pm$ 5.9
16	12 $\pm$ 3.9	36 $\pm$ 4.4	20 $\pm$ 3.1	32 $\pm$ 5.5
28	11 $\pm$ 3.7	54 $\pm$ 4.5	16 $\pm$ 2.6	18 $\pm$ 3.6

4

1 Table 4

2 Clean fleece weight, clean wool growth rate and mean fibre diameter of Merino ewes selected  
 3 either for increased resistance to *H. contortus* or at random (control) and unsupplemented or  
 4 supplemented with 250 g/day cottonseed meal for either the 6 weeks prior to, or the 6 weeks  
 5 after, the start of lambing.

Effect	Clean fleece weight (kg)		Clean wool growth (g/d)				Mean fibre diameter ( $\mu\text{M}$ )			
	Mean	se	Period 1		Period 2		Period 1		Period 2	
			Mean	se	Mean	se	Mean	se	Mean	se
<i>Selection line</i>										
Control	2.5	0.05	6.1	0.16	4.7	0.29	19.4	0.25	18.5	0.50
Resistant	2.3	0.05	5.5	0.17	4.3	0.31	19.1	0.27	18.0	0.50
<b>P value</b>	0.02		0.01		NS		NS		NS	
<i>Pregnancy/Lactation status</i>										
Single	2.5	0.03	6.2	0.11	4.9	0.12	19.4	0.15	18.6	0.16
Twin	2.3	0.06	5.4	0.19	3.7	0.25	19.1	0.27	17.8	0.33
<b>P value</b>	0.01		0.001		0.001		NS		0.05	
<i>Supplement<sup>A</sup></i>										
Nil	2.4	0.06	5.8	0.11	4.4	0.24	19.2	0.28	18.1	0.47
Prepartum	2.4	0.06	6.0	0.11	4.6	0.28	19.1	0.29	18.4	0.55
Post partum	2.4	0.06	6.0	0.11	4.7	0.28	19.6	0.29	18.4	0.55
<b>P value</b>	NS		NS		NS		NS		NS	

6 Period 1 from day -72 to -26 and period 2 from day -25 to 28 in relation to midpoint lambing.

7 Pregnancy status for clean fleece weight and clean wool growth and mean fibre diameter

8 (Period 1). Lactation status for clean wool growth and mean fibre diameter (Period 2).

9 <sup>A</sup> Means are adjusted for the covariate of clean fleece weight.

1 Table 5  
 2 Birth weight, body weight at day 125 and body weight gain from birth to day 125 of lambs  
 3 born to Merino ewes selected either for increased resistance to *H. contortus* or at random  
 4 (control) and unsupplemented or supplemented with 250 g/day cottonseed meal for either the  
 5 6 weeks prior to, or the 6 weeks after, the start of lambing.

Effect	Birth weight (kg)		Body weight day 125 (kg)		Body weight gain (kg)	
	Mean	se	Mean	se	Mean	se
<b>Supplement</b>						
Nil	3.3 <sup>a</sup>	0.07	14.9	0.40	11.6	0.36
Prepartum	3.6 <sup>b</sup>	0.08	15.7	0.40	12.2	0.36
Post partum	3.4 <sup>ab</sup>	0.07	15.7	0.39	12.3	0.35
<b>P value</b>	0.04		NS		NS	
<b>Selection line</b>						
Control	3.6 <sup>a</sup>	0.07	15.4	0.66	11.8	0.58
Resistant	3.3 <sup>b</sup>	0.08	15.6	0.62	12.3	0.55
<b>P value</b>	0.05		NS		NS	
<b>Birth type</b>						
Single	3.8 <sup>a</sup>	0.44	16.5 <sup>a</sup>	0.49	12.8 <sup>a</sup>	0.47
Twin	3.1 <sup>b</sup>	0.52	14.3 <sup>b</sup>	0.36	11.3 <sup>b</sup>	0.35
<b>P value</b>	0.001		0.001		0.001	
<b>Rearing status</b>						
Single			16.7 <sup>a</sup>	0.32	13.2 <sup>a</sup>	0.31
Twin			14.2 <sup>b</sup>	0.58	10.9 <sup>b</sup>	0.55
<b>P value</b>			0.001		0.001	
<b>Parity</b>						
Primiparous	3.3 <sup>a</sup>	0.07	15.9	0.24	12.3	0.23
Multiparous	3.6 <sup>b</sup>	0.03	15.0	0.51	11.8	0.49
<b>P value</b>	0.001		NS		NS	
<b>Gender</b>						
Male	3.5	0.05	15.8 <sup>a</sup>	0.35	12.4	0.34
Female	3.4	0.05	15.0 <sup>b</sup>	0.37	11.7	0.36
<b>P value</b>	NS		0.05		NS	

6 Means within column and main effect with different superscripts differ significantly with P  
 7 value shown in table. NS, not statistically significant.

1 Fig 1. Timing of experimental events relative to lambing mid-point which is defined as day 0  
2 on 15<sup>th</sup> September 1999. Numbers indicate start and end day for each activity. T, Tracer  
3 sheep grazed in plots. Pre, period of prepartum supplementation. Post, period of postpartum  
4 supplementation.

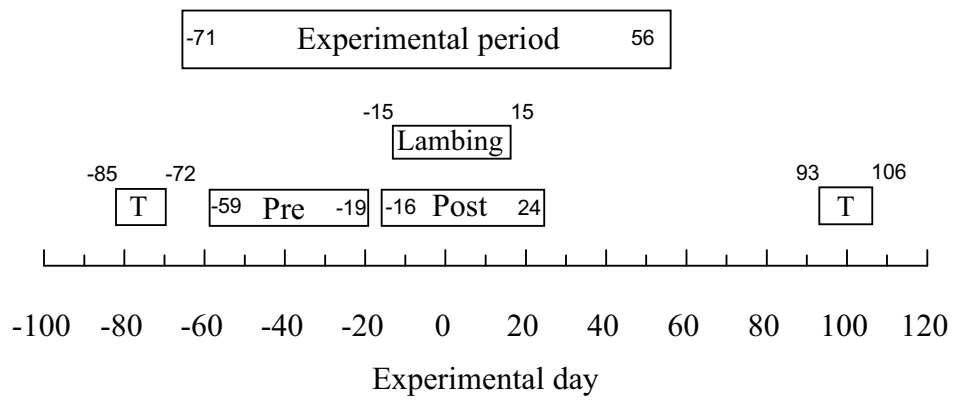
5

6 Fig. 2. Faecal egg counts (epg) (backtransformed least square means) of R (open symbols and  
7 dashed lines) and C (filled symbols and solid line) Merino ewes which were either  
8 unsupplemented (circle) or supplemented with 250g CSM/day for 6 weeks prior to (square) or  
9 6 weeks after (triangle) the start of parturition. Note: significant differences ( $P<0.05$ )  
10 between nil fed v's pre and post partum supplementation at days 16 and 28.

11

12 Fig 3: Main effect of supplementation on body weight (adjusted least squares mean  $\pm$  pooled  
13 se) of R (open symbols and dashed lines) and C (filled symbols and solid line) Merino ewes  
14 which were either unsupplemented (circle) or supplemented with 250g CSM/day for 6 weeks  
15 prior to (square) or 6 weeks after (triangle) the start of parturition. Note: significant ( $P<0.01$ )  
16 differences between nil fed v's prepartum supplementation at days -42 and -26 and significant  
17 ( $P<0.05$ ) differences between nil fed v's pre and post partum supplementation at day 28.

18

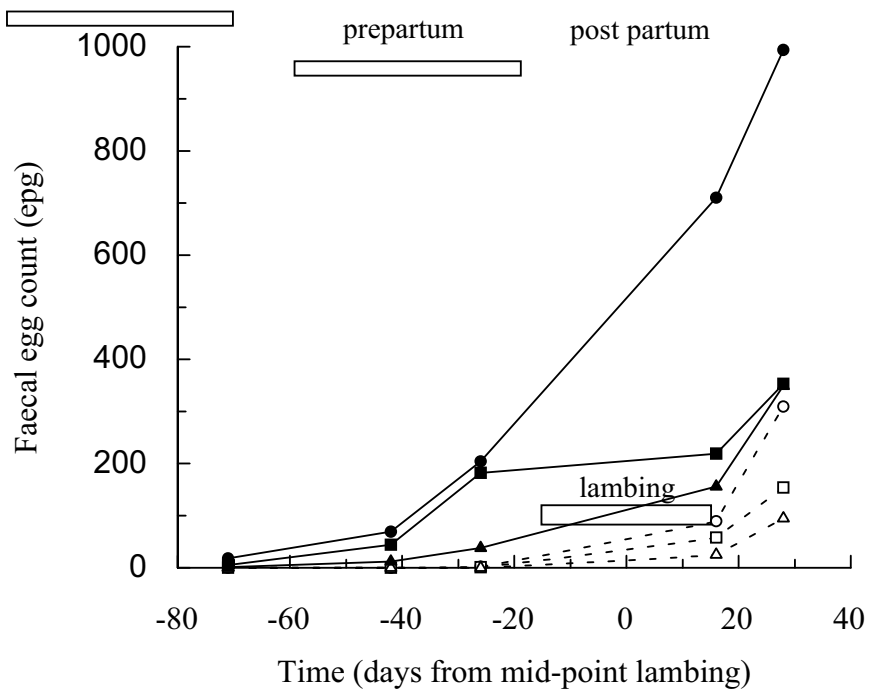


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2

3 Fig.1. Kahn et al.

1

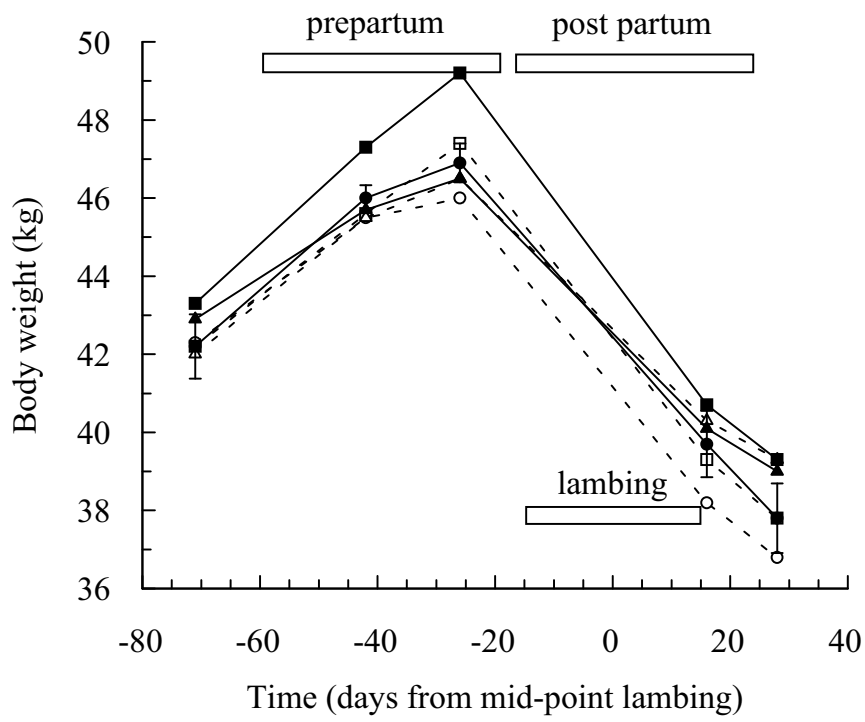


2

3 Fig. 2. Kahn et al.

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1



2

3

4 Fig 3: Kahn et al.

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