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Nutritional management to reduce embryo mortality in short-term flushed ewes

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Project Investigators

The studies presented as part of the current project were conducted by researchers at Charles Sturt University and the NSW Department of Primary Industries (Wagga Wagga Agricultural Institute) University through the Graham Centre for Agricultural Innovation, which is a strategic alliance between NSW DPI and CSU. The following researchers contributed significantly to the design and conduct of the studies and analysis or interpretation of results.

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Abstract

High levels of embryo mortality reduce the number of lambs born and therefore returns to sheep producers, and there is concern that lucerne pasture may increase embryo mortality. Estimated embryo mortality and/or fetal numbers were compared in two studies in which the timing (in relation to conception) and quantity of lucerne available differed. Embryo mortality was not increased in artificially inseminated ewes fed in pens at maintenance levels of lucerne compared with ewes fed a low-protein pellet, but the proportion of pregnant ewes bearing multiple fetuses was reduced if ewes were fed lucerne *ad libitum* compared with at maintenance (0.21 versus 0.33) during early pregnancy. This contrasts with a grazing study which showed grazing naturally cycling ewes on ample live lucerne (estimated energy intake at least 1.8 times maintenance) for 7 days before and to either day 7 or throughout joining increased fetal numbers compared with ewes only grazing dead pasture (1.6 versus 1.3 fetuses/ewe, respectively; lambs marked/ewe joined 115% versus 96%, respectively), with no increase in non-pregnant ewes or returns to service. The implications are that producers may achieve large increases in lambing percentages by grazing naturally cycling ewes on lucerne pasture before and during joining, but until further information is known, artificially inseminated ewes should be fed at maintenance levels.

Executive Summary

Increased rates of embryo mortality reduce the number of potential lambs born, in turn potentially reducing profits for producers. The rate of embryo mortality is known to be increased by diets high in protein or nitrogen (McEvoy *et al.* 1997; Meza-Herrera *et al.* 2010), and at high levels of energy intake (Cumming *et al.* 1975; Parr *et al.* 1987). The reduction can be substantial, with pregnancy rate reduced from 68% at maintenance feeding to 48% at twice maintenance feeding in the study of Parr *et al.* (1987). This knowledge has led to industry concerns that grazing lucerne (*Medicago sativa*) pasture at joining may reduce reproductive performance.

In contrast, grazing studies show that flushing ewes with lucerne or other live pasture for seven days before ovulation increases ovulation rate (King *et al.* 2010) and grazing lucerne for 7 days before and the first 7 days of an autumn joining increased the number of fetuses per ewe joined by 18% (Robertson *et al.* 2014b). In the latter study the ewes were removed from lucerne at day 7 of joining since ewes appear most sensitive to high levels of nutrition at days 11 and 12 of pregnancy due to reduced progesterone levels causing embryo mortality (Parr 1992). However, this practice reduces the proportion of ewes which mate on a flushed ovulation. Grazing lucerne further into joining would allow a larger proportion of ewes to be flushed, but it is not clear whether this would result in more lambs born, or less due to the higher intake over the sensitive period increasing the level of embryo mortality.

A pen-feeding study was conducted during 2013 and 2014 to evaluate whether the level of feeding and time of removal from lucerne could alter estimated embryo mortality and fetal numbers, so lambs born per ewe. This study included two replicates of five treatments, and used 176 (2013) or 216 (2014) oestrus synchronised and artificially inseminated Merino ewes. The ewes were fed daily between days -7 and day 17 after insemination either an energy maintenance ration of a commercial pellet, or freshly cut irrigated lucerne pasture at *ad libitum* quantities. Lucerne *ad libitum* was also fed between days 0 and 7 and 0 and 17 to evaluate the effect of time of removal from lucerne, while lucerne was also fed at maintenance levels between day 0 and 17. Blood samples were taken on days -8, 5, 12 and 17, and embryo mortality was estimated as the difference between the number of corpora lutea and fetal numbers.

As a main effect, feeding lucerne at *ad libitum* quantities reduced the proportion of pregnant ewes with multiple fetuses (0.21) compared with ewes fed at maintenance levels (0.33), and similarly reduced the proportion of ewes estimated to retain all embryos (0.21 compared with 0.37). Feeding lucerne *ad libitum* to day 17 resulted in similar proportion with multiples (0.18) as feeding only to day 7 (0.22). Reproductive performance was similar for ewes fed maintenance levels of either lucerne or pellets. The proportion of non-pregnant ewes was similar between all treatments. The proportion of female lambs born was lower for ewes fed lucerne *ad libitum* before insemination (0.24) compared with all other treatments (0.45 to 0.53). Plasma urea concentrations were not associated with the proportion of ewes with multiples. The proportion of ewes pregnant was positively associated with plasma progesterone concentrations at days 5 and 12.

A grazing study was conducted in 2014 to determine whether flushing with lucerne and grazing lucerne to day 7 or throughout joining would result in similar fetal numbers under normal commercial conditions. This study included two replicates of three treatments. Naturally cycling Merino ewes (300) grazed dead pasture before and during joining, or lucerne from 7 days before to day 7 of joining, or lucerne before and throughout a 36 day joining. The proportions of ewes returning to service and pregnant were similar between treatments. Grazing lucerne to day 7 increased fetal numbers per ewe joined compared with dead pasture

(1.60 compared with 1.31), but grazing lucerne throughout joining was not different to removing ewes at day 7. Grazing lucerne resulted in 115% lambs marked/ewe joined, compared with 96% if grazing dead pasture only. The sex ratio of lambs was similar between treatments. Plasma urea concentration was elevated and progesterone reduced by grazing lucerne.

The contrasting results between the pen (artificially inseminated) and grazing study (natural joining) imply different recommendations are required for the different situations. The grazing study achieved its objective by demonstrating that autumn-joined, naturally cycling ewes can be grazed on lucerne pasture for 7 days before and throughout joining without risking lower reproductive performance. Additionally, there was no further benefit of continuing to graze lucerne past day 7 of joining, so if quality feed is limited, the lucerne is most efficiently used by removing ewes at this time. The sheep industry can benefit from adoption of these practices, since the study shows there is substantial potential for producers to increase their lambing percentages by flushing ewes on lucerne, thereby likely increasing profitability. Benefits are also likely if ewes were flushed on other live pastures, since previous studies indicate the response is not specific to lucerne, although the level of response will vary depending on the quality and quantity of feed available.

The results of the pen study indicate that it was not lucerne *per se*, but rather above-maintenance levels of feeding with artificially inseminated ewes which appeared to increase the level of embryo mortality. Feeding lucerne at *ad libitum* levels only to or past day 7 post-insemination produced a similar reproductive outcome, comparable with the grazing study. The industry can benefit from the recommendation to feed artificially bred ewes at maintenance levels during early pregnancy through minimising the risk of reproductive failure, until more information is known. The cause of the higher level of embryo mortality in artificial situations is not clear, and the artificial breeding, the feeding method used or both may have contributed. Further research in this area is recommended so producers who artificially breed sheep or are hand-feeding sheep have improved evidence-based practical guidelines by which to optimise reproductive efficiency and profitability. Additionally, work is ongoing to determine under what conditions flushing with lucerne may alter the sex ratio of lambs, and whether there are longer-term effects of flushing with lucerne on the performance of the progeny.

Table of Contents

1	Background.....	8
2	Projective Objectives	8
3	Methodology	9
3.1	Pen experiment.....	9
3.2	Grazing experiment.....	11
4	Results.....	12
4.1	Pen study.....	12
4.1.1	Feed intake	12
4.1.2	Ewe condition score and live weight	14
4.1.3	Embryo mortality and reproductive performance.....	15
4.1.4	Blood parameters	18
4.2	Grazing study.....	21
4.2.1	Pastures	21
4.2.2	Ewe weight and condition	22
4.2.3	Reproductive performance.....	23
4.2.4	Blood parameters	25
4.2.5	Wool production.....	26
5	Discussion	26
5.1	Pen study.....	26
5.2	Grazing study.....	27
5.3	Differing responses in pen and grazing studies.....	28
5.4	Further implications.....	29
5.5	Achievement of project objectives.....	30
6	Conclusions/Recommendations.....	31
7	Key Messages	32
7.1	Grazing naturally cycling ewes on live lucerne pasture before and during joining increases lambs born	32
7.2	Feed artificially bred ewes at maintenance levels in early pregnancy to maximise lambs born	32
8	Acknowledgements.....	32
9	Bibliography	33
10	Appendix	38
10.1	Communication activities	38

10.1.1	Appendix 1.....	39
10.1.2	Appendix 2.....	42
10.1.3	Appendix 3.....	43
10.1.4	Appendix 4.....	46
10.1.5	Appendix 5.....	47
10.1.6	Appendix 6.....	48

1 Background

Increased weaning rates are one means of increasing the profitability of sheep production (Warn *et al.* 2006). The number of lambs born per ewe can be increased by improved nutrition (flushing), with a short period of feeding targeting days 10-14 of the oestrous cycle, sufficient to increase ovulation rates (Stewart and Oldham 1986). The number of fetuses per ewe was increased by 18% in naturally cycling autumn-joined ewes by grazing lucerne (*Medicago sativa*) pasture for 7 days before and 7 days into joining, rather than dead pasture (Robertson *et al.* 2014b). This time period restricts the proportion of ewes which mate on a flushed ovulation, but it is not clear whether continuing to graze lucerne throughout joining would increase or decrease fetal numbers due to the risk of embryo mortality.

Embryo mortality can be increased by feeding ewes at twice their energy maintenance requirement, with pregnancy rates reduced from 68% to 48% in comparison with maintenance feeding (Parr *et al.* 1987). This response may be due to a reduction in circulating concentrations of progesterone, with the sheep embryo appearing to be most sensitive to reductions in progesterone on days 11 and 12 post mating (Parr 1992). Embryo mortality is also known to increase with high intake of protein or nitrogen (McEvoy *et al.* 1997; Bishonga *et al.* 2006). This knowledge has led to industry concern that grazing lucerne pasture during joining will result in increased levels of embryo mortality, resulting in a reduction in the number of lambs born per ewe.

The aim of this study was to establish whether fetal numbers per ewe differ for ewes consuming lucerne for different time periods and in different quantities during the peri-conceptual period. This knowledge would allow the development of evidence-based recommendations for flushing strategies to assist producers to improve the reproductive performance of their sheep flocks.

2 Projective objectives

The objectives of the project were to have, by 31 January 2015:

1. Determined, from a pen-feeding experiment (360 ewes in total), whether the level of fresh lucerne pasture consumed during early pregnancy increases embryo mortality.
2. Determined, from a pen-feeding experiment, whether retaining ewes on high levels of lucerne after 1 week of pregnancy increases embryo mortality.
3. Determined, from a pen-feeding experiment, whether fresh lucerne pasture, compared with a maintenance pellet, consumed during early pregnancy increases embryo mortality.
4. Determined, from a pen-feeding experiment, whether fresh lucerne pasture in the week before mating increases embryo mortality.
5. Developed and provided guidelines to industry identifying a safe level of intake of lucerne during early pregnancy.
6. Developed and provided guidelines to industry for nutritional management of ewes at joining to optimise the number of lambs born.

3 Methodology

3.1 Pen experiment

A report of this experiment has been accepted for publication in *Animal Reproduction Science* (Robertson *et al.* in press).

All procedures were approved by the Animal Care and Ethics committee of Charles Sturt University (project number 12/105).

The experiment was conducted during 2013 and 2014 at Charles Sturt University, Wagga Wagga, in southern New South Wales. Five treatments were evaluated in a randomised block design with two replicates of each treatment. The treatments were (Day 0 = day of artificial insemination and M = energy requirement for maintenance):

1. Control – Day -7 to 17 commercial pellet (low grain/protein) at 1.0 M
2. Day 0 to 17 fresh lucerne pasture *ad libitum*
3. Day 0 to 17 fresh lucerne pasture at 1.0 M
4. Day 0 to 7 fresh lucerne pasture *ad libitum*
5. Day -7 to 17 fresh lucerne pasture *ad libitum*

Ewes were fed the pellet at maintenance levels for the duration of pen feeding (23 January to 3 March 2013 and 16 January to 24 February 2014, Days -22 to 17) except if being fed lucerne. The pellet was based on faba bean and oat hulls (Fibre pellet; Conqueror Milling Company, Cootamundra). All ewes had access to a mineral lick, containing no urea, for the duration of pen feeding to avoid mineral deficiencies.

Mixed age Merino ewes (n = 176 in 2013, 216 in 2014) were stratified on age, condition score and weight then randomly allocated to one of ten outdoor pens (6 x 8m) on Day -22 to allow a two week period of adaptation prior to differential feed treatments. Oestrous cycles were synchronised using CIDR inserts (Eazi-breed CIDR sheep and goat; Pfizer Australia Pty Ltd, West Ryde, Australia) for 14 days, and 200iu Pregnecol serum gonadotrophin (Bioniche Animal Health (A/Asia) Pty Ltd, Armidale, Australia) was injected on CIDR removal. The ewes were artificially inseminated two days later on Day 0 (14 February 2013 and 7 February 2014) by a commercial contractor using laparoscopy and fresh semen from Merino rams. Rectal ultrasound was used on Day 8, 9 or 10 after insemination to determine the number of corpora lutea, as a measure of ovulation rate per ewe. The ewes were removed from pens on Day 18 after insemination, and run as one mob in a paddock of dry pasture, supplemented with pellets over the next 2 weeks to maintain liveweight. In 2013, rams were run with the ewes between Days 29 and 50 with the aim of impregnating ewes not pregnant from artificial insemination. Trans-abdominal ultrasound was used to determine fetal numbers on Day 57 (2013) or Day 60 (2014), with only pregnancies resulting from artificial insemination included in data analysis. All treatment groups lambed within the same paddock. The lambs born each day were identified to their dam, tagged, weighed and their sex and survival to marking were recorded. Similar numbers of ewes per treatment

lambled in 2013 (between 26 and 30 ewes; 74 to 91%), minimising the risk of treatments influencing production in 2014.

Blood samples were collected from 8 ewes per pen by venipuncture before differential feeding (on Day -8 in 2013 and Day -9 in 2014), then on days 5, 12 and 17 after insemination. The same ewes were sampled on each day within years. Sampling commenced at 12 noon on each day, with samples stored on ice until plasma was separated by centrifuge. The plasma samples were frozen and stored at -20°C until evaluated for urea, ammonia (2013 only), progesterone and leptin concentrations by commercial laboratories. The ewes were weighed before the start of differential feeding, then at days 8 and 18 after insemination. Condition score was recorded at day -8 and 18 and prelambling.

Irrigated lucerne pasture was cut daily with a mower, and a sample collected for measurement of dry matter. A similar weight of lucerne was taken from each daily sample of dried lucerne, and bulked for periods of up to seven sequential days for analysis of nutritive value. Feed refusals were collected daily and dry weights recorded. The lucerne samples were analysed by the Feed Quality Service of NSW Department of Primary Industries (Wagga Wagga, New South Wales) for crude protein (CP), neutral detergent fibre (NDF), dry matter digestibility (DMD), and digestibility of organic matter (DOMD). Values were estimated using near infrared spectroscopy (NIR) and metabolisable energy (ME) calculated using the equation $ME = 0.203 \text{ DOMD (\%)} - 3.001$ (Anon. 2007). Samples of the pellets were analysed using wet chemistry methods by the same laboratory.

Data was analysed using Genstat® 16th edition (VSN International 2013). Data for five ewes was removed from the 2013 data set – two were found to be pregnant at insemination, one was not inseminated due to immature ovaries, and two were found to have ovarian cancer or a cyst at ultrasound. Five other ewes died throughout the year, most prior to lambing. In 2014, two ewes were removed due to illness during pen-feeding, one was not inseminated due to severe mastitis, while two ewes died from pregnancy toxemia during the lambing period due to a severe weather event. Ewe condition score and weight were analysed using linear mixed modelling with repeated measurements, with value at allocation, treatment and year as fixed effects and tag, pen and year as random effects. Categorical data (embryo losses, lamb survival, proportion of ewes with multiple ovulations and foetuses, proportion female lambs) were analysed using generalised linear mixed modelling using a binomial distribution. The standard errors for backtransformed means were not available from this analysis due to the logit transformation. Treatment x year was used as the fixed effect and pen as the random effect. Progesterone (after transformation by natural logarithm) leptin and urea concentrations were analysed using linear mixed models with concentration at Day -8 as a co-variate, with pregnancy status, treatment, day of sampling and year as the fixed effects and tag, pen and year as random effects. Interactions were removed from the model when not significant ($P > 0.05$). Relationships between pregnancy status or fetal number and progesterone or urea at each day of sampling were tested using simple linear regression. Means were considered to differ significantly if $P \leq 0.05$.

3.2 Grazing experiment

All procedures were approved by the Animal Care and Ethics committee of Charles Sturt University (project number 13/088).

A grazing experiment was conducted during 2014 on a commercial property (34°48'S; 147°26'E) 40 km north of Wagga Wagga. The design included two replicates of three treatments, where Day 0 = day of ram introduction:

1. Ewes grazing senescent (dead) pasture from Day -7 to 36
2. Ewes grazing lucerne pasture from Day -7 to 7
3. Ewes grazing lucerne pasture from Day -7 to 36

Mature Merino ewes ($n = 300$) were stratified on age (3.5 and 5.5 yrs) then randomly allocated to six groups (50 per group) and placed on appropriate pastures on Day -7. Merino rams (2 per paddock) fitted with crayon harnesses were added to each paddock on Day 0 (7 March). Non-experimental ewes were added to appropriate paddocks to maintain similar numbers of ewes per paddock throughout joining. The rams were rotated between treatments within replicates on Days 3, 7, 12, 14, 21, and 28. Crayon marks were recorded on Days 3, 7, 14, 21, 28 and 36 of joining, with crayon colour changed on Day 14 to allow returns to service to be detected. From the end of joining (Day 36) all ewes grazed together. Fetal number and age were determined by transabdominal ultrasound 52 days after rams were removed. The ewes lambled in two paddocks, one for each replicate, to avoid paddock effects on treatments. Paddocks were checked usually twice daily during lambing and new-born lambs assigned to ewes, tagged and sex recorded. Survival of lambs to marking and weight at marking were recorded.

The ewes were weighed and condition scored on Days -7, 7, 21 and 36. Eight ewes per group (16 per treatment) were randomly selected from those which were crayon marked by Day 3, and blood samples were collected from these same ewes on Days 3, 7 and 14 of joining for analysis of plasma urea and progesterone concentrations. The methods used were as for the pen experiment.

Pasture herbage mass (live and dead) was recorded weekly throughout the experiment using the method of Haydock and Shaw (1975). Samples for dead herbage quality were taken at the same time using the 'toe-cut' method (Cayley and Bird 1996), while for live quality, pluck samples of live pasture by species (lucerne, witch grass (*Panicum capillare*), annual grass (mainly barley grass or brome grass) were collected for each paddock, when sufficient quantities were available, and dried. The proportion leaf and stem of live lucerne was recorded for lucerne samples cut to ground level along the transect used for herbage mass, after drying and weighing. The 'toe-cut' samples of dead pasture, pluck samples of live lucerne pasture and pluck samples of annual grass, if available, for each paddock on Days -8, 6, 13 and 27 of joining were analysed by the Feed Quality Service of NSW Department of Primary Industries (Wagga Wagga, New South Wales) as for the pen study. The intake of pasture was estimated using GrazFeed using GrazFeed version 4.1.13 (Freer *et al.* 1997).

Pasture data was not analysed. Sheep data were analysed using Genstat® 16th edition (VSN International 2013). One ewe was removed from the experiment as she became ill during joining. Three other ewes died before or during lambing; where lambs were not yet born the fetuses were examined to determine sex but their data was not used to calculate lamb survival. Categorical data were analysed using generalised linear mixed modelling using a binomial distribution, with treatment as the fixed and replicate as the random effects. The standard errors for backtransformed means were not available from this analysis due to the logit transformation. For the joining period, ewe weight and condition score data were analysed using repeated measures; pre-lambing ewe data and lamb weights were analysed separately using linear mixed modelling. The number of lambs born and marked per ewe joined was analysed using linear mixed modelling. Associations between fetal number or pregnancy status and blood parameters were tested using linear regression. The differences between treatments in plasma variables was evaluated: Urea and progesterone data were logarithmically transformed prior to analysis using repeated measures, with treatment x Day x pregnancy status as the fixed effects, ewe tag as the subject and Day as the time point. Means were considered to differ significantly if $P \leq 0.05$.

4 Results

4.1 Pen study

4.1.1 Feed intake

The estimated nutritive value of the pellets and lucerne are shown in Table 1. The estimated protein intake of ewes fed pellets at maintenance was generally lower than that of ewes fed lucerne at maintenance, while ewes fed lucerne *ad libitum* consumed up to five times the protein of those fed pellets (Table 2). Ewes fed lucerne at *ad libitum* levels had an estimated energy intake up to 200% that of the control in 2013, but only up to 160% in 2014 between Days 1 and 17.

Table 1 Mean nutritive value of pellets and lucerne pasture (\pm sem) in 2013 and 2014.

	Pellet		Lucerne pasture	
	2013	2014	2013	2014
Dry matter (%)	95	95	25 \pm 0.2	20 \pm 0.1
Neutral detergent fibre (% of DM)	65	50	51 \pm 1.4	47 \pm 2.0
Acid detergent fibre (% of DM)	40	27	34 \pm 0.9	32 \pm 1.0
Crude protein (% of DM)	6.7	8.8	18.2 \pm 0.43	21.2 \pm 2.32
Digestibility of dry matter (%)	57	54	62 \pm 0.9	66 \pm 2.9
Digestible organic matter in the dry matter (%)	56	54	60 \pm 0.8	63 \pm 2.3
Metabolisable energy (MJ/kg DM)	9.1	8.8	9.1 \pm 0.16	9.7 \pm 0.46
Crude Fat (% of DM) ^A	1.8	1.6	-	-

^ACrude fat was not determined for lucerne samples.

Table 2 Estimated mean (\pm sem) daily energy (MJ ME) and crude protein (g) intake of ewes during pen-feeding 2013 and 2014. Intakes on the day of insemination (Day 0) are not included.

Year	Days of Experiment	Control	0 to 17 M	0 to 7 <i>ad libitum</i>	0 to 17 <i>ad libitum</i>	-7 to 17 <i>ad libitum</i>
<i>Energy intake (MJ ME/day)</i>						
2013	-7 to -1	7.8 \pm 0.0	7.8 \pm 0.0	7.8 \pm 0	7.8 \pm 0	10.9 \pm 0.47
	1 to 7	7.8 \pm 0.0	9.1 \pm 0.20	13.7 \pm 0.50	14.2 \pm 0.54	15.6 \pm 0.57
	8 to 17	7.8 \pm 0.0	7.3 \pm 0.30	7.8 \pm 0	14.1 \pm 0.61	15.4 \pm 0.79
2014	-7 to -1	10.5 \pm 0.0	10.5 \pm 0.0	10.5 \pm 0	10.5 \pm 0	12.4 \pm 0.98
	1 to 7	10.5 \pm 0.0	8.9 \pm 0.52	14.6 \pm 0.64	13.9 \pm 1.03	16.8 \pm 0.94
	8 to 17	10.5 \pm 0.0	8.9 \pm 0.52	10.5 \pm 0	15.4 \pm 1.45	15.5 \pm 1.40
<i>Protein intake (g/day)</i>						
2013	-7 to -1	58 \pm 0.0	58 \pm 0.0	58 \pm 0.0	58 \pm 0.0	221 \pm 9.6
	1 to 7	58 \pm 0.0	174 \pm 4.0	259 \pm 9.4	271 \pm 9.9	294 \pm 10.8
	8 to 17	58 \pm 0.0	147 \pm 6.3	58 \pm 0.0	283 \pm 13.2	315 \pm 15.0
2014	-7 to -1	105 \pm 0.0	105 \pm 0.0	102 \pm 0.0	105 \pm 0.0	251 \pm 17.7
	1 to 7	105 \pm 0.0	188 \pm 9.5	354 \pm 15.5	291 \pm 25.3	410 \pm 23.0
	8 to 17	105 \pm 0.0	208 \pm 13.1	130 \pm 0.0	361 \pm 34.2	334 \pm 28.7

4.1.2 Ewe condition score and live weight

The condition score and fasted weight of ewes at allocation (Day -22) was similar between treatments (3.1 and 2.7, 55.6 and 49.9 kg in 2013 and 2014, respectively). The unfasted weight of ewes at the start of differential feeding (Day -8) did not differ between treatments, but by Day 18 ewes fed lucerne at *ad libitum* levels to Day 17 were heavier than ewes in all other treatments (Fig. 1). Ewes in all treatments lost weight between fasted weights at allocation and Day 18 in 2013, although ewes fed lucerne at *ad libitum* levels for 17 days lost less weight (≤ 3.8 kg) than ewes fed maintenance levels of lucerne or pellets (≥ 5.8 kg). In 2014, ewes in treatments fed lucerne at *ad libitum* levels to Day 17 gained at least 3.9 kg, while all other treatments lost up to 2.6 kg weight.

The condition score of ewes declined between Days -8 and 18 for ewes in the 0-17 maintenance and 0-17 *ad libitum* lucerne treatments in 2013 (Fig. 2). In 2014, condition score declined for ewes in the Control and 0-17 maintenance treatments.

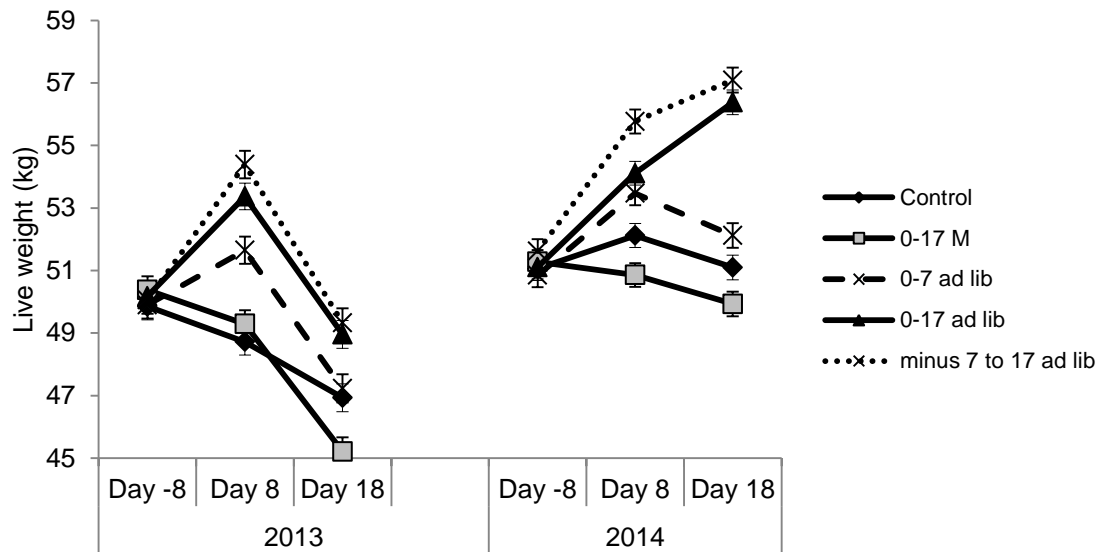


Fig. 1. Mean live weight (kg ± sem) of ewes in five dietary treatments at Days -8, 8 (unfasted) and 18 (fasted) in 2013 and 2014, using fasted weight at allocation as a covariate.

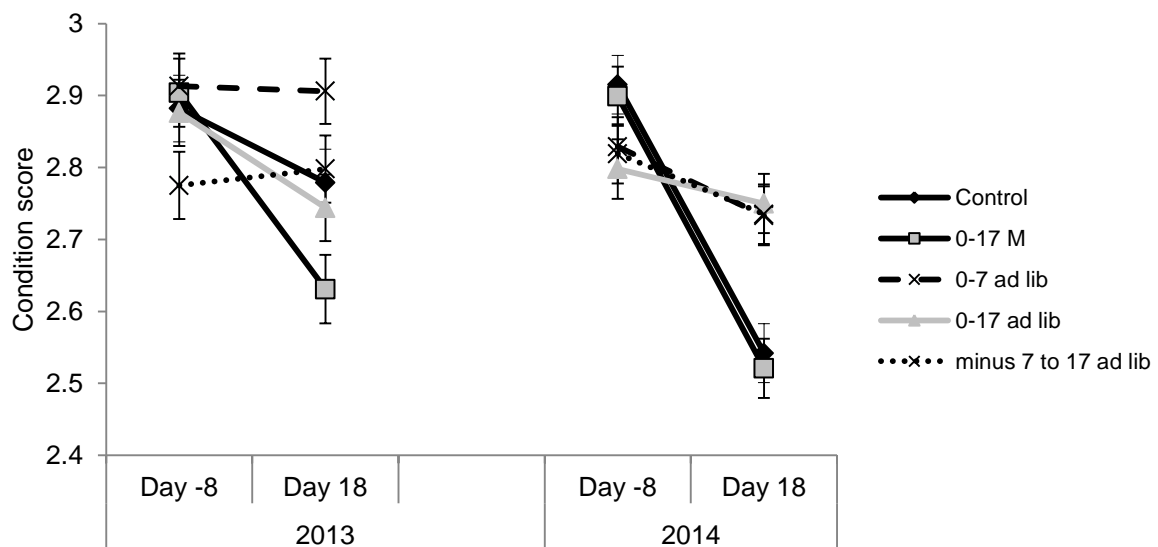


Fig. 2. Mean condition score (± sem) of ewes in five dietary treatments at Days -8 and 18 in 2013 and 2014, using condition score at allocation as a covariate.

4.1.3 Embryo mortality and reproductive performance

The proportion of ewes with multiple ovulations was not increased ($P = 0.07$) by feeding lucerne before insemination, compared with the control (Table 3). The proportion of ewes pregnant was similar between treatments, but the proportion of pregnant ewes carrying multiple fetuses was reduced by feeding lucerne *ad libitum* between Day 0 and 17 after insemination compared with ewes fed either pellets or lucerne at maintenance levels. When

analysed as a main effect, the proportion of pregnant ewes carrying multiple fetuses was reduced by feeding lucerne *ad libitum* for any length of time after insemination, with the number of fetuses per ewe reduced by 0.12.

Estimated embryo mortality was increased for ewes fed lucerne at *ad libitum* levels compared with control or maintenance feeding. As a main effect, the proportion of ewes retaining all embryos was lower ($P=0.002$) for ewes fed *ad libitum* compared with at maintenance (0.21 cf. 0.37, respectively) and the proportion with partial loss of embryos was higher ($P=0.033$) (0.37 cf. 0.26, respectively). When comparing between treatments (Table 4), the proportion of ewes retaining all embryos was halved in ewes fed lucerne *ad libitum* from Day -7 to 17 in comparison with maintenance feeding.

The number of lambs born or marked per ewe and lamb survival were similar between treatments (Table 5). The birthweight of lambs was similar between treatments, but the proportion of lambs born which were female was lower ($P=0.31$) if the ewes were fed lucerne for 7 days before insemination compared with all other treatments.

Table 3 Mean reproduction outcomes for ewes fed a control diet or lucerne fed at maintenance or *ad libitum* around the time of artificial insemination (2013 and 2014).

Reproduction parameter ^A	Dietary Treatment					<i>P</i> value	Main effect of level of feeding		
	Control	0-17 M	0-7 <i>ad lib</i>	0-17 <i>ad lib</i>	Minus 7-17 <i>ad lib</i>		Maintenance	<i>Ad lib</i>	<i>P</i> value
Ovulation rate per ewe (all ewes)	1.67 ± 0.082	1.73 ± 0.082	1.74 ± 0.081	1.85 ± 0.083	2.04 ± 0.083	0.113	1.70 ± 0.068	1.87 ± 0.056	0.084
Proportion of ewes with multiple ovulations	0.65	0.67	0.67	0.77	0.83	0.070	0.66	0.76	0.045
Proportion of ewes pregnant	0.67	0.62	0.57	0.68	0.59	0.687	0.65	0.62	0.505
Proportion of all ewes with multiple fetuses	0.23	0.20	0.13	0.14	0.13	0.426	0.21	0.13	0.043
Proportion of pregnant ewes with multiple fetuses	0.34 b	0.32 b	0.22 ab	0.18 a	0.23ab	0.026	0.33	0.21	0.001
No. fetuses/ewe	0.90 ± 0.081	0.83 ± 0.080	0.71 ± 0.080	0.81 ± 0.081	0.73 ± 0.081	0.337	0.87 ± 0.060	0.75 ± 0.049	0.146
No. fetuses/pregnant ewe	1.34 ± 0.062	1.32 ± 0.064	1.23 ± 0.067	1.18 ± 0.062	1.23 ± 0.067	0.347	1.33 ± 0.044	1.21 ± 0.038	0.046

a,b: Different letters within rows within analyses indicate means differ significantly (P<0.05).

Table 4 Mean estimated embryo losses for ewes fed a control diet or lucerne fed at maintenance or *ad libitum* around the time of artificial insemination (2013 and 2014).

Embryo losses (proportion)	Control	0-17 M	0-7 <i>ad lib</i>	0-17 <i>ad lib</i>	Minus 7-17 <i>ad lib</i>	<i>P</i> value
<i>Total retention</i>	0.36 b	0.38 b	0.24 ab	0.22 ab	0.17 a	0.031
<i>Partial loss</i>	0.29	0.23	0.30	0.43	0.37	0.095
<i>Total loss</i>	0.33	0.37	0.43	0.31	0.42	0.527

a,b: Different letters within rows within analyses indicate means differ significantly ($P < 0.05$).

Table 5 Mean lambing performance and lamb survival for ewes fed a control diet or lucerne fed at maintenance or *ad libitum* around the time of artificial insemination (2013 and 2014).

Reproductive parameter	Control	0-17 M	0-7 <i>ad lib</i>	0-17 <i>ad lib</i>	Minus 7-17 <i>ad lib</i>	<i>P</i> value
Lambs born/pregnant ewe	1.32 ± 0.066	1.30 ± 0.067	1.17 ± 0.071	1.22 ± 0.065	1.22 ± 0.071	0.506
Lambs born/ewe inseminated	0.80 ± 0.092	0.79 ± 0.092	0.62 ± 0.091	0.77 ± 0.092	0.66 ± 0.092	0.523
Proportion lamb survival (marked/lamb born)	0.73	0.7	0.78	0.82	0.68	0.689
Lambs marked/ewe inseminated	0.59 ± 0.083	0.55 ± 0.083	0.48 ± 0.082	0.64 ± 0.083	0.47 ± 0.084	0.583
Lambs marked/pregnant ewe	0.92 ± 0.085	0.89 ± 0.087	0.86 ± 0.091	0.98 ± 0.084	0.83 ± 0.092	0.791
Proportion female lambs	0.47 b	0.45 b	0.53 b	0.50 b	0.24 a	0.031

4.1.4 Blood parameters

Plasma urea concentrations were higher for ewes fed lucerne than those fed pellets, but urea concentrations were not always higher for ewes fed lucerne *ad libitum* compared with lucerne at maintenance (Fig. 3). Fetal numbers were not associated with urea concentrations. Plasma ammonia concentrations were similar between treatments (data not shown).

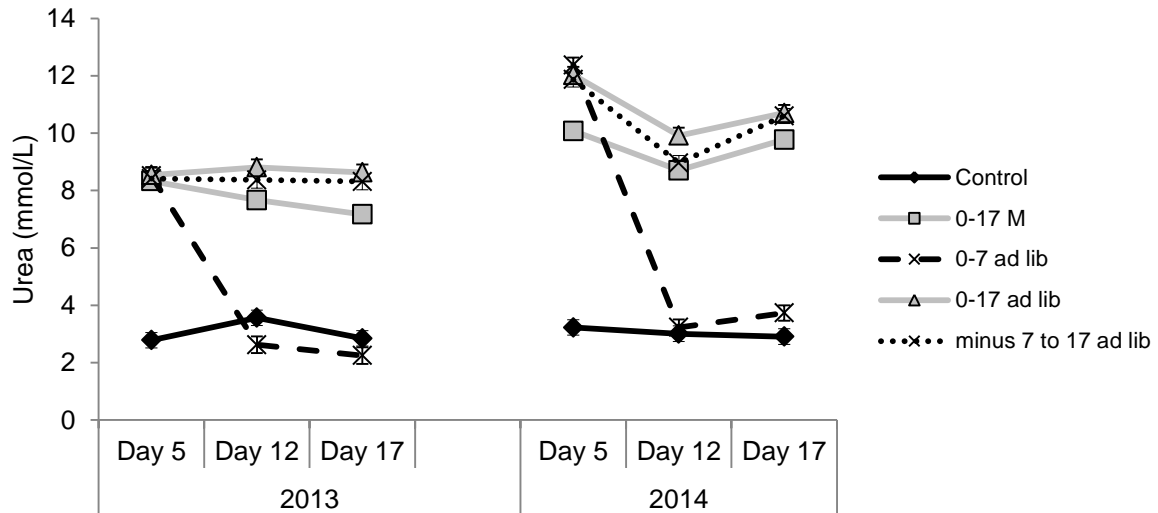


Fig. 3. Mean plasma urea concentration (mmol/L \pm sem) at Days 5, 12 and 17 after artificial insemination in different dietary treatments in 2013 and 2014.

The plasma progesterone concentrations of pregnant ewes were similar between treatments on each of the days sampled. In non-pregnant ewes, progesterone concentrations were lower on Day 17 for ewes fed lucerne compared with Control ewes, and lower at Day 12 if ewes were fed lucerne *ad libitum* from before insemination (Fig. 4).

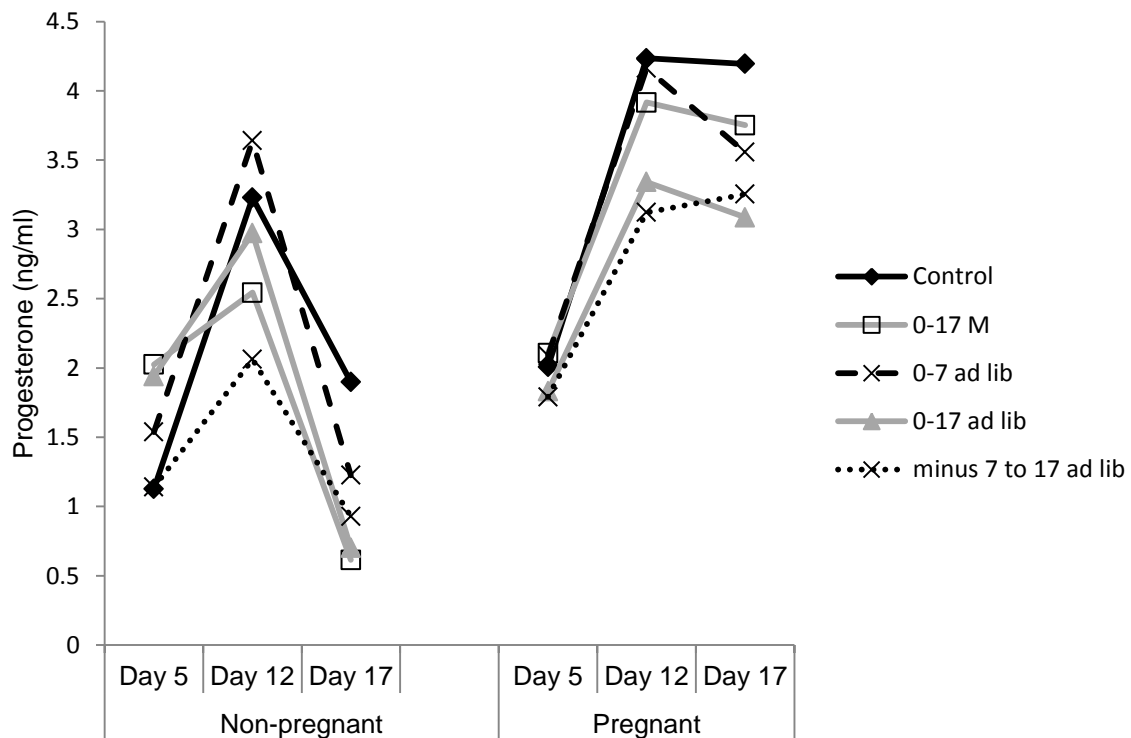


Fig. 4. Mean plasma progesterone concentration (ng/ml) for non-pregnant and pregnant ewes at Days 5, 12 and 17 after artificial insemination in different dietary treatments.

Progesterone concentrations did not increase to the same degree between Days 5 and 12 for ewes fed lucerne at *ad libitum* levels to Day 17 (up to 1.29 ng/ml) compared with Control ewes (2.11 ng/ml). Pregnancy rates were lower for ewes with progesterone concentrations of 1 ng/ml or less on Day 5 in comparison with ewes with progesterone concentrations of 1.5 to 2 ng/ml or higher (Fig. 5). The number of fetuses per ewe was larger at higher progesterone concentrations on Day 5 ($r^2 = 0.15$) and Day 12 ($r^2 = 0.16$).

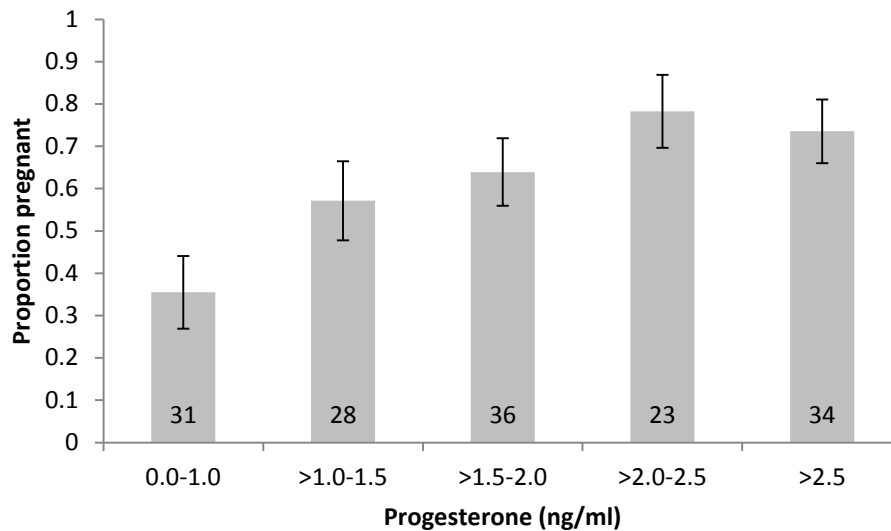


Fig. 5. The proportion of ewes (\pm sem) pregnant for different levels of plasma progesterone at Day 5 after insemination (2013 and 2014). Numbers in columns indicate number of ewes.

Plasma leptin concentrations were lowest in ewes fed lucerne at maintenance levels, but there were no interactions between treatment and pregnancy status or day of sampling. The mean leptin concentrations of pregnant and non-pregnant ewes are shown in Fig. 6.

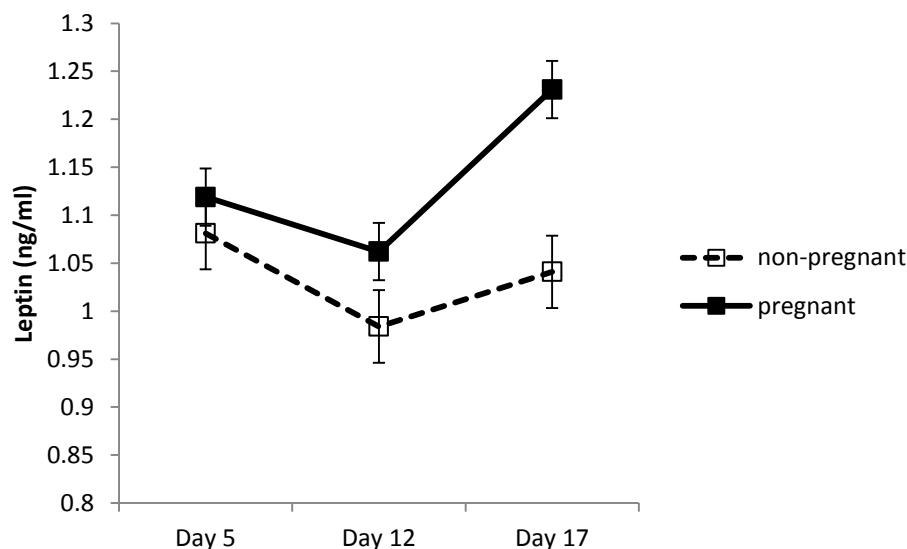


Fig. 6. Mean (\pm sem) plasma leptin concentration (ng/ml) for non-pregnant and pregnant ewes at Days 5, 12 and 17 after insemination (2013 and 2014).

4.2 Grazing study

4.2.1 Pastures

The mean quantity of dead pasture available in each paddock (senescent and lucerne paddocks) was generally between 1500 and 4000 kg DM/ha on all sampling occasions. Senescent pastures contained a small quantity of live herbage due to germination of annual grasses, which increased at the end of joining (Fig 7.). At least 400 kg DM/ha live pasture was available in lucerne paddocks except on Day 0 of joining, with over 2000 kg/ha available by the end of joining. A high proportion of the live lucerne was leaf, which remained between 40 and 65% of live herbage throughout joining. The minimum mean height of live lucerne was 22 cm across all days of sampling. The estimated nutritive value of live lucerne was relatively high compared with dead pasture (Table 6).

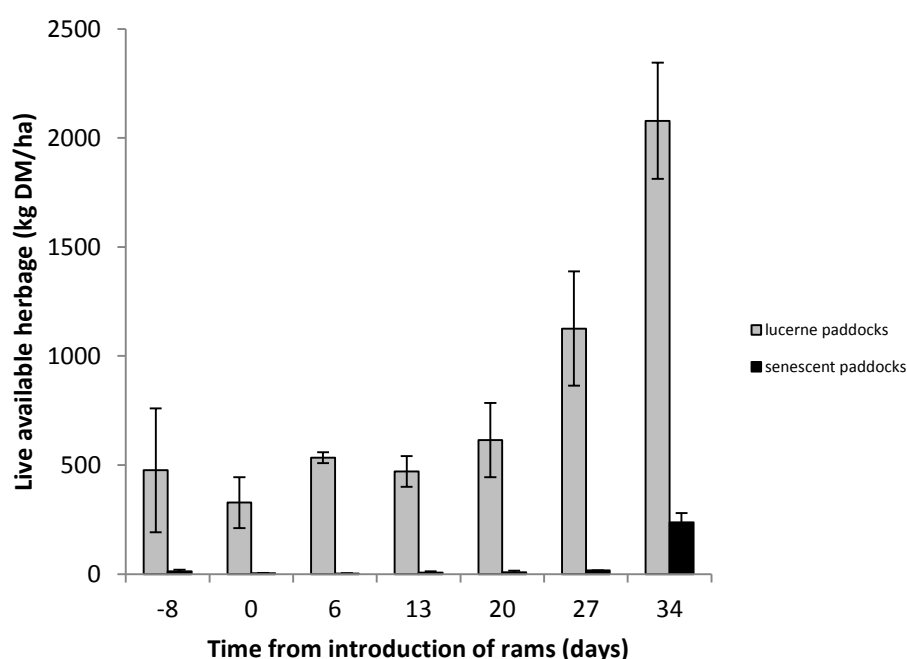


Fig. 7. Mean quantity of live available herbage (kg DM/ha \pm sem) in lucerne and senescent pasture paddocks prior to and following the introduction of rams (Day 0).

Table 6 Metabolisable energy (MJ ME/kg DM), crude protein (CP %), neutral detergent fibre (NDF %) and water soluble carbohydrate (WSC %) of lucerne and dead grass pasture between Day -8 and 27 from introduction of rams (Day 0) (mean \pm sem).

	Treatment	Plant type	Day-8	Day 6	Day 13	Day 27
ME (MJ/kg DM)	Lucerne	Live lucerne	11.3 \pm 0.65	14.6 \pm 0.20	12.6 \pm 0.10	14.1 \pm 0.05
	Senescent	Dead grass	5.8 \pm 0.25	5.4 \pm 0.15	5.3 \pm 0.15	5.4 \pm 0.25
CP (% of DM)	Lucerne	Live lucerne	19.9 \pm 1.15	32.6 \pm 0.65	32.9 \pm 2.60	31.8 \pm 1.70
	Senescent	Dead grass	2.2 \pm 0.20	<2.0	<2.0	2.7 \pm 0.65
NDF (% of DM)	Lucerne	Live lucerne	37.0 \pm 2.00	27.5 \pm 0.50	30.5 \pm 0.50	30.5 \pm 1.50
	Senescent	Dead grass	77 \pm 0.50	81 \pm 1.00	80 \pm 2.00	79 \pm 2.00
WSC (% of DM)	Lucerne	Live lucerne	3.4 \pm 0.45	3.4 \pm 0.15	2.5 \pm 0.15	7.6 \pm 1.53
	Senescent	Dead grass	1.2 \pm 0.19	2.3 \pm 1.29	2.1 \pm 1.29	2.0 \pm 0.98

4.2.2 Ewe weight and condition

The mean weight (54.1 kg) and condition score (2.8) of ewes at Day -7 was similar in all treatments (Fig. 8). The weight of ewes grazing senescent pasture was maintained between Day 7 and 36 of joining, while weight gains of up to 300 g/day were recorded for ewes which grazed lucerne throughout joining, so that ewes grazing lucerne throughout were 10 kg heavier than ewes grazing senescent pasture at the end of joining. The mean condition score of ewes was maintained between Day -7 and 36 for ewes grazing senescent pasture, although there was a 0.1 decline in condition between Day -7 and 7. Mean condition score increased to 3.7 by the end of joining for those ewes grazing lucerne throughout.

The GrazFeed estimated intake of metabolisable energy was 1.8 to 1.9 times maintenance requirement while ewes grazed lucerne. The estimated energy intake for ewes grazing dead pasture was 0.8 of maintenance requirement up to Day 7 of joining, and 1.6 to 1.7 times maintenance thereafter.

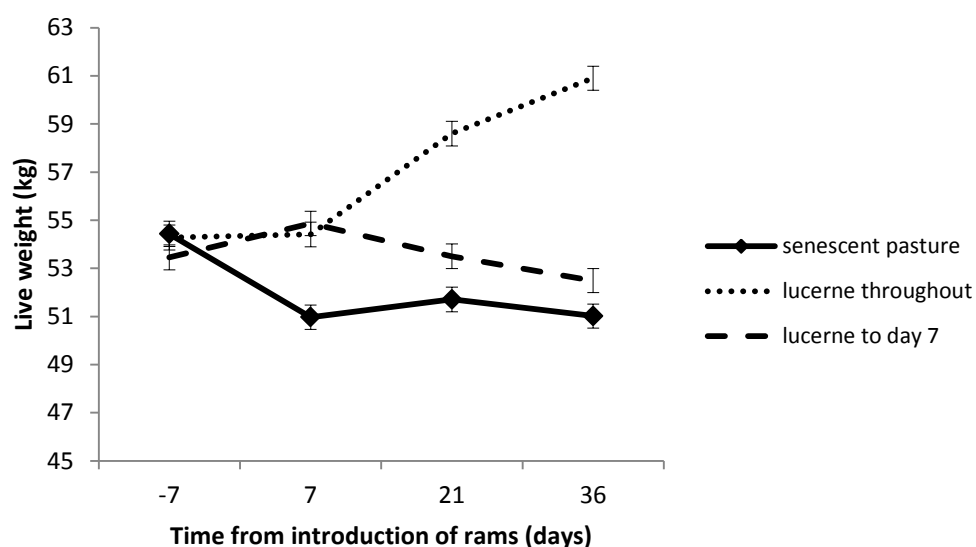


Fig. 8. Mean unfasted live weight ($\text{kg} \pm \text{sem}$) of ewes in three treatments prior to and following the introduction of rams (Day 0).

4.2.3 Reproductive performance

The reproductive performance of ewes is shown in Table 7. The majority (> 80%) of ewes were marked by rams and assumed to be mated during the first 14 days of joining in all treatments. The proportion of ewes which returned to service, and the proportion of ewes which were pregnant, were similar between treatments. The proportion of ewes joined carrying multiple fetuses was higher for ewes which grazed lucerne compared with senescent pasture, such that the number of fetuses per ewe joined was 30% higher. Reproductive performance was similar for ewes which were removed from lucerne at Day 7 of joining and those which grazed lucerne throughout joining.

The number of lambs born per ewe joined was at least 30% higher for ewes which had grazed lucerne compared with those grazing only senescent pasture (Table 8). Lamb survival to marking was similar between treatments, although the survival of singles (82%) and twins (75%) was higher than that of triplets (47%). The weight of twin born lambs which died was similar between treatments. Insufficient singles died for comparison. The proportion of lambs born which were female and the weight of lambs at marking was similar between treatments. The number of lambs marked per ewe joined was 18 or 19% higher for ewes which had grazed lucerne compared with those that had not.

Table 7 Mean (\pm sem) reproductive performance of ewes in three treatments.

	Senescent pasture	Lucerne throughout	Lucerne to Day 7	P- value
Proportion raddled to Day 14	0.87	0.92	0.83	0.172
Proportion returning to service	0.19	0.15	0.19	0.684
Proportion non-pregnant	0.12	0.06	0.09	0.341
Proportion with multiple foetuses of ewes joined	0.43 a	0.67 b	0.65 b	0.001
No. fetuses per ewe joined	1.31 a \pm 0.095	1.61 b \pm 0.095	1.60 b \pm 0.095	0.002

a,b: Different letters within rows indicates means differ significantly ($P < 0.05$).

Table 8 Mean (\pm sem) production of lambs born to ewes in three treatments.

	Senescent pasture	Lucerne throughout	Lucerne to Day 7	P- value
Lambs born per ewe joined ^A	1.30 a \pm 0.070	1.67 b \pm 0.069	1.63 b \pm 0.70	<0.001
Proportion lamb survival ^A	0.78	0.74	0.74	0.685
No. lambs marked per ewe joined	0.96 a \pm 0.060	1.14 b \pm 0.060	1.15 b \pm 0.060	0.040
Marking weight (kg)	15.3 \pm 0.43	15.0 \pm 0.40	14.6 \pm 0.41	0.509
Dead weight of twins (kg)	5.1 \pm 0.55	4.7 \pm 0.52	4.7 \pm 0.53	0.977
Proportion female lambs	0.42	0.54	0.53	0.178

a,b: Different letters within rows indicates means differ significantly ($P < 0.05$).

^ALambs born is calculated from scanned fetal number where actual lambs born was not observed (ewe didn't lamb or abandoned lambs before tagging); Lamb survival excludes 12 lambs which could not be identified to ewes and therefore treatment.

4.2.4 Blood parameters

Fetal numbers and pregnancy outcome were not associated with glucose, progesterone or urea concentrations or the degree of change in progesterone levels between Days 3 and 14 of joining.

The mean plasma urea concentration was higher ($P<0.001$) while ewes grazed lucerne compared with dead pasture (Fig. 9) on each of the days sampled. Progesterone concentrations increased between Day 3 and 14 of joining, and were higher ($P=0.002$) for ewes grazing dead pasture than for ewes grazing lucerne. The increase in progesterone between Days 3 and 7 and 3 and 14 of joining were similar between treatments.

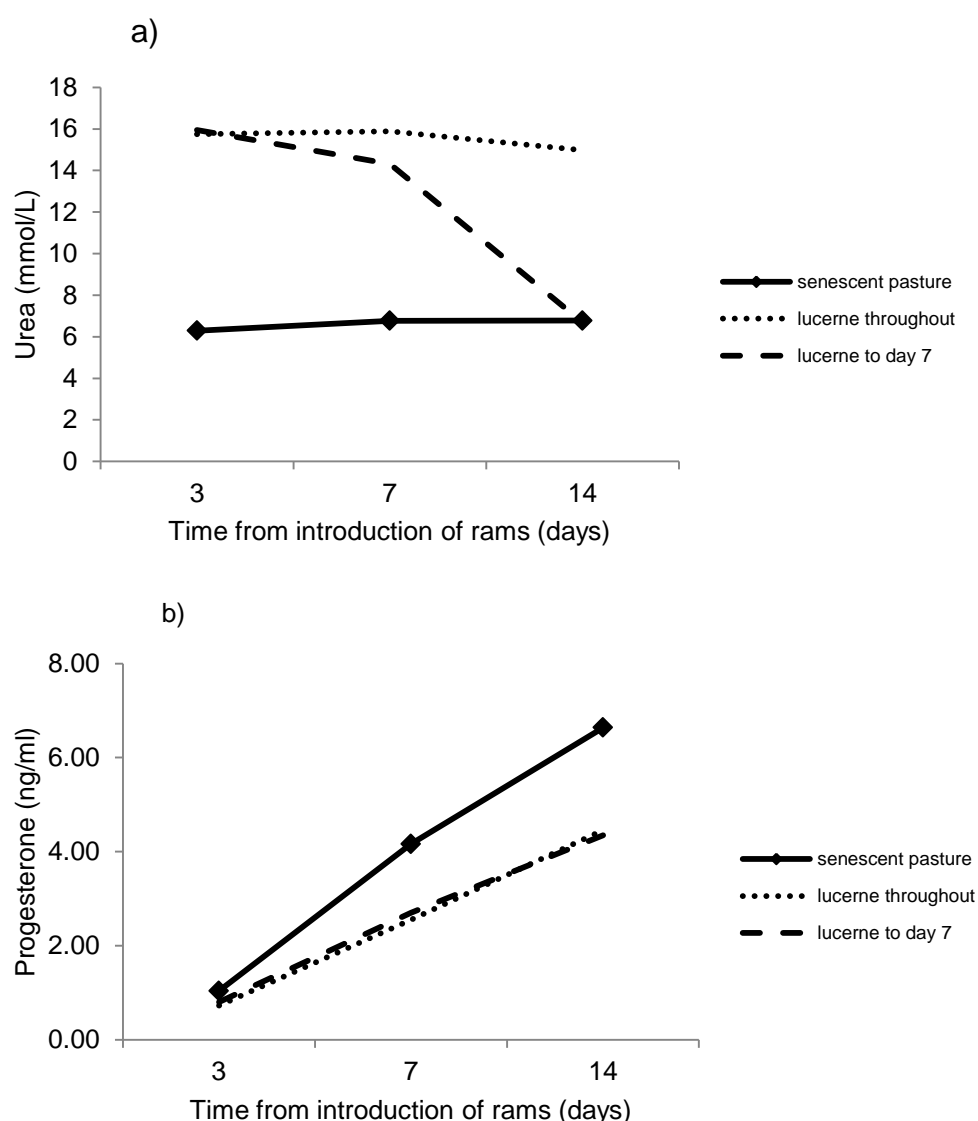


Fig. 9. Mean plasma a) urea concentration (mmol/L) and b) progesterone concentration (ng/ml) for ewes in three treatments on Days 3, 7 and 14 after introduction of rams. Means are backtransformed.

4.2.5 Wool production

The quantity and quality of wool produced was similar between treatments (Table 9). However, fleece weights tended ($P=0.057$) to be higher if ewes grazed lucerne throughout joining (5.1 kg) compared with to Day 7 (4.8 kg) or only senescent pasture (4.7 kg). Staple strength also tended ($P=0.07$) to be higher for ewes in the senescent pasture treatment (34 N/ktex) compared with the other treatments (≤ 31 N/ktex).

Table 9 Mean (\pm sem) wool characteristics of ewes.

	Mean
Greasy fleece weight (kg)	4.8 \pm 0.06
Yield (%)	75 \pm 0.4
Fibre diameter (microns)	19.6 \pm 0.48
Staple length (mm)	72 \pm 0.8
Staple strength (N/ktex)	32 \pm 0.7
Minimum along-staple fibre diameter (microns)	17.9 \pm 0.35
Maximum along-staple fibre diameter (microns)	21.7 \pm 0.51

5 Discussion

This project has shown that feeding fresh lucerne pasture at *ad libitum* levels during early pregnancy reduced fetal numbers in artificially inseminated ewes fed in a controlled environment. In contrast, no reduction in lambs born resulted from grazing lucerne for longer than the first 7 days of a natural joining, and flushing on live lucerne produced a 30% increase in fetal numbers compared with grazing senescent pasture. This indicates a need for nutritional management to differ in order to optimise lamb numbers in the two situations. This study supports previous findings that fetal numbers can be substantially increased through grazing naturally cycling autumn-joined ewes on lucerne pasture for 7 days before joining and the first 7 days of joining (Robertson *et al.* 2014b), but extends our knowledge by demonstrating that fetal numbers may not be reduced if lucerne is grazed throughout joining.

5.1 Pen study

The adverse effect of *ad libitum* feeding on fetal numbers in the pen study is consistent with previous studies (Parr *et al.* 1987). Similar fetal numbers resulted from feeding at *ad libitum* levels during the first 7 days or the first 17 days after insemination, and although fetal numbers were not statistically reduced by feeding *ad libitum* during the first 7 days compared with at maintenance, they may have been if larger numbers of ewes had been used. Days 11 and 12 after insemination have previously been reported (Parr 1992) as the period when

twice maintenance feeding is most likely to cause embryo mortality in ewes, which implies that if high feeding levels end prior to this, embryo mortality may be avoided. The current pen results suggest that mortality was not avoided, possibly because high levels of nutrition can alter progesterone concentrations before day 7 of pregnancy and the rate of increase in progesterone prior to day 11, both of which also influence embryo mortality in sheep (Ashworth *et al.* 1989) and cattle (Diskin *et al.* 2012). In addition, Parr (1992) only evaluated days 9 to 13.

Increased embryo mortality can be associated with a reduction in circulating plasma progesterone concentrations (Parr 1992), due to high feed intake causing an increased blood flow and clearance rate via the liver (Parr *et al.* 1993). It is possible that this may be the mechanism which caused fetal numbers to be reduced by *ad libitum* feeding in the current pen study. Progesterone levels were reduced 17 days after insemination if ewes were fed lucerne, although this only occurred in non-pregnant ewes, and the rate of increase in progesterone was also reduced by *ad libitum* feeding. A larger number of ewes sampled in each fetal class may have improved the precision of measurement and assisted the identification of trends.

Unlike some studies (McEvoy *et al.* 1997; Bishonga *et al.* 2006), high nitrogen intake resulting in high plasma urea levels did not appear to be the means by which fetal numbers were reduced in the pen study. Plasma urea levels were not consistently higher in ewes fed lucerne *ad libitum* compared with at maintenance, and fetal numbers were not associated with urea concentrations. The concentrations of urea in ewes fed lucerne at maintenance were above the levels which have been shown to be associated with increased embryo mortality (Bishonga *et al.* 2006), yet mortality was similar to Control ewes.

In a recent review, Valezquez (2011) suggested that embryo mortality is unlikely to result in ruminants which are not in severe negative energy balance, even where plasma urea levels are high. Ewes fed lucerne at *ad libitum* levels for 17 days lost less weight in 2013 and gained more weight in 2014 than ewes fed at maintenance. The reduction in fetal numbers observed in ewes fed lucerne *ad libitum* to Day 17 compared with at maintenance therefore appears unrelated to the levels of weight gain or loss observed. It is likely that much of the weight loss observed in 2013 occurred in the period allocation to Day -8, when ewes were adapting to pen conditions and the ration was reduced according to the estimated energy content of the pellets. The ration was increased on Day -7 when weight loss indicated that a higher energy intake was required. The fact that condition score was maintained between Days -8 and 18 in the lucerne *ad libitum* Day -7 to 17 treatment and only reduced by 0.2 in the lucerne *ad libitum* Day 0 to 17 supports the possibility that ewes were not in severe negative energy balance during the period of differential feeding (Days -8 to 18) in 2013.

5.2 Grazing study

Grazing naturally cycling ewes on large quantities of lush lucerne pasture or senescent pasture produced similar proportions of non-pregnant ewes and ewes returning to service. Similar fetal numbers were also obtained whether ewes were removed from lucerne at day 7 or remained throughout joining, indicating grazing lucerne did not result in lower reproductive performance. Any increase in embryo mortality associated with higher ovulation rates (Ashworth *et al.* 1989) which could be expected as a result of flushing did not result in a net

reduction in fetal numbers compared with unflushed ewes grazing senescent pasture. The higher ovulation rates expected due to flushing ewes on lucerne (King *et al.* 2010), evident in the current study by higher rates of multiple fetuses, combined with no apparent large increase in embryo mortality, meant flushing ewes on lucerne was effective at increasing the number of lambs born per ewe joined.

The quality of the lucerne was high, as indicated by its leafiness and the energy and protein content, and the gains in liveweight and condition support the estimate of energy intake at 1.9 times maintenance for ewes grazing lucerne. This is similar to the twice maintenance levels of intake which have been associated with embryo mortality and a 19% reduction in pregnancy rate in other studies (Parr *et al.* 1987). The energy intake for ewes grazing dead pasture may be overestimated, when compared with actual weight and condition score, probably associated with the difficulty in estimating the small quantity of live pasture, and difficulty in sheep accessing pasture germinating through a large bulk of senescent herbage. However, the estimates of weight change also include some measurement error, since unfasted weights were used, and the degree of weight loss recorded to day 7 of joining for ewes grazing senescent pasture is likely to be an overestimate given ewes appeared to maintain weight thereafter. While condition score did decline between day -7 and 7, this may be measurement error given the subjective nature of the method, given the ewes grazing senescent pasture maintained body condition over the joining period.

5.3 Differing responses in pen and grazing studies

The differing response in fetal numbers in the pen and grazing studies for ewes given access to large quantities of lucerne is important in developing guidelines for producers breeding ewes. The cause of the difference cannot be determined from these studies, but there are several possibilities.

The estimated level of intake of lucerne in the grazing study was similar or above that which caused a reduction in fetal numbers in the pen study, and was sufficient to reduce progesterone levels, but there was no evidence of an increase in embryo mortality. Additionally, the plasma urea levels of ewes consuming lucerne in the grazing study were higher than those in the pen study, but there was no indication that these impaired reproductive performance.

There was no indication that the reduction in fetal numbers in the pen study was specific to lucerne since similar reproductive performance was achieved when ewes were fed maintenance levels of lucerne or pellets. In addition, there was no evidence of increased embryo mortality (pregnancy rates, returns to service) in the grazing study when ewes were also apparently consuming large quantities of lucerne (based on live weight gains). Previous studies which indicated an increase in embryo mortality for ewes grazing lucerne compared with grass pastures did not rotate rams between treatments (Smith *et al.* 1979), so in that study poor ram performance may have been the cause. Reductions in fetal numbers due to grazing lucerne in early reports were due to a reduction in ovulation rates associated with coumestrol levels (Scales *et al.* 1977; Smith *et al.* 1979), so grazing lucerne heavily infected with aphids or fungus should be avoided. High coumestrol levels are unlikely to be the cause of the lower fetal numbers in the pen study because numbers were reduced when ewes were only fed lucerne after insemination.

Abnormal embryo development is known to increase with use of gonadotrophins (Moor *et al.* 1985), such that the synchronisation/insemination procedure may have contributed to the adverse impact of nutrition on fetal numbers in the pen study. This may be unlikely, since a low rate of gonadotrophin was used, but the lowest rate which has an adverse impact is unknown. However, the oestrous synchronisation procedure, through manipulation of progesterone levels, still has potential to interact with nutritional effects on progesterone levels to alter embryo mortality.

The differing feeding methods used may also have contributed to the differing impact on fetal numbers, either alone or by interaction with breeding method. Some toxins found in feedstuffs are known to cause embryo mortality (McEvoy *et al.* 2001), such that it is possible that mowing and feeding lucerne once daily under hot conditions may have promoted toxin formation. This could have occurred in ewes fed *ad libitum*, but not at maintenance, since maintenance rations were consumed in a shorter time period. Additionally, the once daily feeding, even where feed was constantly available in *ad libitum* treatments, may have had an effect. Plasma ammonia levels vary more over time with a post-feeding peak in response to high nitrogen intake much more evident than for plasma urea levels (Bishonga *et al.* 2006). However, more frequent blood sampling than used in the current study would be required to detect such responses.

The proportion of female lambs was reduced by feeding ewes lucerne *ad libitum* for 7 days before insemination. Further studies are required to establish whether this is repeatable under a range of commercial management conditions, since a higher proportion of males may be a financial advantage in prime lamb and ram breeding enterprises, whereas female lambs may be preferred in self-replacing flocks to allow a higher culling rate and genetic gain. The proportion female lambs was not reduced, over the entire drop, by grazing ewes on lucerne rather than senescent pasture for 7 days before joining. Blood samples from both studies are currently being analysed to determine whether the fatty acid profile was altered by lucerne, and whether this was related to embryo mortality and sex of lamb. Dietary differences in fatty acids consumed by ewes are known to determine the sex ratio of lambs (Gulliver *et al.* 2013; Clayton *et al.* in press), but further information is required to allow management guidelines for producers to be developed.

5.4 Further implications

The quantity, fibre diameter or staple strength of wool produced by ewes in the grazing study was not altered by flushing on lucerne, and under the seasonal conditions experienced, no supplementary feeding of ewes was required. However, the trend for lower staple strength and higher fleece weight may have been significant if a larger number of ewes had been used, so should be considered in Merino enterprises. Twin-bearing ewes have higher nutritional requirements than single-bearing ewes, so flushing may increase both the need for supplementary feeding, and the risk of producing wool of lower staple strength in poorer seasonal conditions. Although the effects on lamb survival and growth to marking were minimal in both the pen and grazing studies, under poorer nutritional conditions twin-born lambs may be disadvantaged. Producers are advised to consider the management of twin-bearing ewes and twin-born lambs when evaluating the benefit of increasing twinning rates.

Nutrition around mating and early pregnancy can impair the health of progeny (Ashworth *et al.* 2009), so it is important that no adverse effect has been observed to date. Additionally, nutrition around mating appears to influence the adult reproductive performance of the progeny (Clayton 2014). It is planned to monitor the progeny to determine whether any longer-term effects of flushing with lucerne occur.

Improved methods for measuring embryo mortality are required. The need to artificially inseminate ewes by laparoscope meant laparoscopy could not then be used to detect the number of corpora lutea in the pen study. Rectal ultrasound was the alternative, but its accuracy is limited by the skill of the operator, and there is a lack of skilled operators in Australia. The ovulation rates and embryo mortality reported in this study are likely to be overestimates, due to large follicles in addition to corpora lutea being counted. However, this does not alter the interpretation of the pen study, since fetal numbers were reliable, given their similarity to the number of lambs born.

5.5 Achievement of project objectives

1. Determined, from a pen-feeding experiment (360 ewes in total), whether the level of fresh lucerne pasture consumed during early pregnancy increases embryo mortality.

This objective was met using 392 ewes. The results of the pen study indicate a higher rate of embryo mortality and a reduction in the proportion of pregnant ewes with multiples if ewes are fed *ad libitum* compared with at energy maintenance between day 0 and 17 after insemination.

2. Determined, from a pen-feeding experiment, whether retaining ewes on high levels of lucerne after 1 week of pregnancy increases embryo mortality.

This objective was met. The results of the pen study indicate no difference in the reproductive performance of ewes if lucerne is fed at *ad libitum* levels for 7 days or 17 days after insemination. This finding is supported by the grazing study where grazing lucerne throughout joining or removing ewes from lucerne after Day 7 of joining resulted in similar fetal numbers and proportions of ewes not pregnant or returning to service.

3. Determined, from a pen-feeding experiment, whether fresh lucerne pasture, compared with a maintenance pellet, consumed during early pregnancy increases embryo mortality.

This objective was met. The pen study demonstrated no difference in estimated embryo mortality or other reproductive performance if ewes were fed at maintenance levels of lucerne compared with a maintenance pellet.

4. Determined, from a pen-feeding experiment, whether fresh lucerne pasture in the week before mating increases embryo mortality.

This objective was met. The pen study indicates no difference in estimated embryo mortality for ewes fed lucerne *ad libitum* for 7 days before insemination and for 17 days after insemination, compared with those only fed lucerne for 17 days after insemination. While the low twinning rate in this study may have made it difficult to detect differences, the similar pregnancy rates and returns to service in the grazing study support the finding that any increase in embryo mortality due to either a higher

ovulation rate or high quantities of lucerne pre-mating did not eliminate the benefit of flushing on the number of lambs born.

5. Developed and provided guidelines to industry identifying a safe level of intake of lucerne during early pregnancy.

This objective was met. Recommendations have been provided to industry via scientific and producer publications, presentations, and provision of detailed reports to extension providers (see Appendices). Safe levels of intake are *ad libitum* for naturally cycling ewes grazing lucerne pasture, but maintenance levels of feeding for artificially inseminated ewes, until further information is known.

6. Developed and provided guidelines to industry for nutritional management of ewes at joining to optimise the number of lambs born.

This objective was met. The grazing study showed a 30% increase in the number of lambs born, and an 18% increase in the number of lambs marked per ewe joined if naturally cycling, autumn-joined ewes were grazed on lucerne pasture for 7 days before joining and the first 7 days of joining. Ewes could be grazed on lucerne throughout joining without any impact on the number of lambs born, but if less ewes mated and fell pregnant during the first 2 weeks of joining, potentially the number of lambs born could increase if ewes remained on lucerne after day 7 of joining. The implication is that where the availability of lucerne is limited, ewes only need to graze lucerne for 14 days to achieve a large increase in the number of lambs born. The level of response will depend on the quality and quantity of lucerne, and on ewe factors. In contrast, it is suggested that artificially inseminated ewes should be fed at maintenance levels post-insemination to optimise the number of lambs born, until further information is known. These guidelines have been published and disseminated to the industry, although this work is continuing.

6 Conclusions/recommendations

Large increases in lambs born per ewe joined could be achieved by sheep producers grazing autumn-joined ewes on lucerne or other live pasture rather than senescent pasture for 7 days before and for the first 7 days of joining. There was no increased risk but no additional benefit if ewes continued to graze lucerne throughout joining. However, increases in fetal numbers may result from continuing to graze lucerne past day 7 of joining if the proportion of ewes mating and falling pregnant in the first 2 weeks of joining is lower than observed in this study. Improvements in lamb numbers were largely associated with increased twinning rates, so producers adopting this practice are advised to consider the higher nutritional requirements of twin-bearing ewes and twin-born lambs to optimise lamb survival and overall production.

An increased risk of embryo mortality occurred if ewes were fed above maintenance requirements for either 7 or 17 days after insemination when oestrus was synchronised and ewes artificially inseminated. It is not clear whether the artificial breeding or feeding methods used (fresh mown, fed once daily), or both, contributed to this outcome. Further research is required to clarify optimum feeding regimes for both naturally cycling and artificially bred ewes to enable producers to optimise lamb production in the range of situations they are likely to encounter across different environments and variable seasons.

Further research is also required to determine the optimum nutritional strategies around mating to alter the sex ratio of lambs.

Integration of the findings of this project into delivery networks such as Best Lamb Best Wool and LifeTime Ewe Management would maximise exposure and potential adoption of recommended joining strategies.

7 Key messages

7.1 Grazing naturally cycling ewes on live lucerne pasture before and during joining increases lambs born

Grazing live, leafy lucerne pasture for 7 days before and for the first 7 days of an autumn joining can increase the number of lambs born by 30%. The level of increase will vary depending on the quantity and quality of lucerne available, and similar gains may be made using other types of live pasture. Where most ewes mate and fall pregnant during the first 14 days of joining, the number of lambs born is unlikely to increase if ewes remain on lucerne throughout joining, so where the availability of lucerne is limited, it may be most efficiently used if ewes are removed after day 7 of joining. Producers joining out of season (before February) need to alter the time of flushing to match peak weeks of mating. Previous research (Scales *et al.* 1977; Smith *et al.* 1979) means producers should avoid grazing lucerne heavily infested with aphids or fungus as this may reduce ovulation rates.

The economic benefit from flushing ewes will depend on the level of response, lamb survival, sheep enterprise and alternative uses for the pasture. Producers can evaluate potential benefits using the EverGraze Green Feed Tactical Management Calculator which is available at www.EverGraze.com.au under Tools.

7.2 Feed artificially bred ewes at maintenance levels in early pregnancy to maximise lambs born

Producers are advised to restrict feeding of artificially bred ewes to maintenance levels after insemination. Embryo mortality appears to increase where ewes are fed *ad libitum*, reducing the number of fetuses per ewe. Maximising the number of fetuses produced through use of recommended feeding levels reduces the cost per lamb born, as well as gaining cost savings from less feed.

8 Acknowledgements

Dr Belinda King contributed to the concept and design for the pen study. Dr Stephanie Knott contributed to the design of the pen study and blood sampling in 2013. Ms Bess Morgan contributed to the conduct, analysis and interpretation of the pen study in 2013. The pen study was made possible through the goodwill and assistance of Mr James Stephens in managing the sheep when not in pens. The grazing study was part-funded by The Australian Wool Education Trust, and numerous students assisted with field work in both studies.

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10 Appendix

10.1 Communication activities

The results have been disseminated to producers through -

Industry publications:

Lawson, P. (2015) A lucerne flush at joining can boost numbers. *Farming Ahead*, March No. 278, 126-128. See Appendix 1.

Shergold, A. (2015) Joining ewes on lucerne. Animal Health Update, March, Local Land Services Riverina. See Appendix 2.

Presentations:

NSW Grassland Research Update, Eurongilly, 25 June 2014. 25 attendees

Graham Centre Sheep Forum at Wagga Wagga, 4 July, 2014. 91 attendees (estimated 73 producers)

Lectures to Animal Science and Veterinary students in subject Ruminant Production. 80 students

Websites:

Project description and key results on the Graham Centre website www.grahamcentre@csu.edu.au . See Appendix 3.

Key results and guidelines forwarded to update the flushing message on the EverGraze website (www.EverGraze.com.au). See Appendix 4.

Communication with extension and industry personnel:

Project results have also been forwarded to Lyndon Kubeil, BestWool co-ordinator and Project Leader of the MLA funded Green Feed Demonstration Project.

Scientific publications:

One conference paper has been published based on the first year results of the pen study (Robertson *et al.* 2014a). See Appendix 5.

One paper has been accepted for publication in Animal Reproduction Science, entitled: Reproductive performance in ewes fed varying levels of cut lucerne pasture around conception (Robertson *et al.* in press). See Appendix 6.

One paper has been drafted, entitled Grazing lucerne throughout joining does not reduce reproductive performance in unsynchronised ewes. See Appendix 7.

10.1.1 Appendix 1



SHEEP PRODUCTION BREEDING MERINOS

Keeping it natural: Grazing large quantities of lucerne during paddock joining can increase the number of lambs born, but attention to pregnant ewe nutrition is required to maximise lamb survival.

Photo: Susan Robertson

A lucerne flush at joining can boost numbers

Joining Merino ewes naturally while they graze lucerne is a highly effective way to increase the number of lambs born. But high levels of lucerne can have the opposite effect on artificially inseminated, pen-fed ewes, as Pamela Lawson discovers.

AT A GLANCE

- ▶ Two recent projects compared the effect of feeding lucerne at different quantities and times to Merino ewes around the autumn joining period.
- ▶ For pen-fed, artificially inseminated ewes, high levels of lucerne fed for the first 17 days of pregnancy may increase embryo mortality.
- ▶ For paddock-mated ewes, grazing lucerne pastures before and over the joining period can increase twinning rates and the number of lambs born.
- ▶ Twinning ewes must be priority managed to maximise lamb survival.

Two studies have recently been conducted to address concerns that joining ewes on lucerne can result in poor reproductive performance. During 2013 and 2014, Meat and Livestock Australia (MLA) funded studies in conjunction with the EH Graham Centre, an alliance between Charles Sturt University and NSW Department of Primary Industries, Wagga Wagga. The studies aimed to address industry concerns that ewes grazing lucerne during joining could cause reproductive issues.

A PEN TRIAL

In both 2013 and 2014, pen trials were conducted using autumn-joined Merino ewes to compare the effect of feeding lucerne pasture at different quantities and

times around the mating period. This is also known as 'flushing'.

The four treatment flocks were fed either fresh lucerne pasture ad libitum from insemination to day seven, fresh lucerne pasture ad libitum from insemination to day seventeen, fresh lucerne pasture at maintenance energy levels from insemination to day 17 or fresh lucerne pasture ad libitum from seven days prior to insemination to day 17. The control flock was fed a commercial maintenance pellet (low protein) at maintenance energy levels.

In each year, the ewes were placed in pens two weeks before feeding began to 18 days after they had been artificially inseminated. Ovulation rates were estimated and blood samples taken on days five, 12 and 17 after insemination, and they

were pregnancy scanned 60 days after insemination.

A RANGE OF RESULTS

For these pen trials, more ewes had more ovulations when fed lucerne for the seven days prior to being inseminated, but this did not affect pregnancy rates compared to the other treatments.

pellets.

Interestingly, the proportion of female lambs born was reduced by feeding lucerne for seven days before insemination, compared to the other treatments. The cause of this is unknown.

WHAT IT MEANS

These results suggest that producers

pregnancy, which may have increased embryo mortality.

The reduced number of female lambs born after feeding lucerne seven days prior to insemination may or may not be desirable to producers, depending on the enterprise they run (for example, ram breeders or prime lamb producers compared to self-replacing flocks).

However, the effects on sex of lambs need to be confirmed with larger numbers and to determine the cause.

Paddock Trials

To validate the pen trial results under normal commercial grazing situations, a second trial was carried out during 2014.

This grazing study was conducted using naturally cycling and mated ewes grazing pasture – typical of management on many commercial farms. The trial involved 300 autumn-joined Merino ewes being allocated to three treatments:

1. Grazing dead grass pasture;
2. Grazing lucerne for seven days before and throughout a 36-day joining period; and
3. Grazing lucerne for seven days before and to day seven of joining before being

The results suggest that producers should avoid grazing or feeding artificially inseminated ewes at high levels of lucerne during early pregnancy.

For those being fed from insemination onwards, feeding lucerne at any rate did not affect pregnancy rates compared to feeding pellets. Feeding maintenance levels of Lucerne gave similar results as maintenance pellets.

However, the proportion of ewes pregnant with twins almost halved if the ewes were fed fresh lucerne pasture ad libitum from insemination to day 17, compared to maintenance levels of either lucerne or

should avoid grazing or feeding artificially inseminated ewes at high levels of lucerne or other feed (1.5-2 times maintenance energy requirements) from insemination to day 17 of pregnancy, to avoid any risk of increased embryo mortality or fertilisation failure. However the high protein intake did not reduce fetal numbers.

The trials showed feeding high levels of lucerne reduced the rate of increase in progesterone levels in ewes during early



Double up: Research has shown flushing paddock-joined ewes on lucerne for two weeks over the joining period will increase twinning rates.

Photo: Susan Robertson

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In contrast: The pen study results indicated that feeding high levels of lucerne to ewes being artificially inseminated actually reduced twinning rates. Photo: Susan Robertson

moved to dead pasture for remainder of joining.

The ewes had ample live, leafy lucerne available – about 500kg per hectare before the start of joining, increasing to 2t/ha by the end of joining.

Intake was estimated to be at least 1.9 times maintenance requirement, so the quantity or quality of the lucerne was unlikely to be limiting.

After joining, all the ewes grazed together.

proportion of non-pregnant ewes or in ewes returning to service when they grazed lucerne, rather than dead pasture, indicating no major issues with embryo mortality or fertilisation failure.

The proportion of female lambs born was similar between treatments.

KEEPING IT NATURAL

This grazing trial showed that for naturally cycling autumn-joined ewes, grazing lucerne for seven days before and during the first

removed from the paddock and the lucerne used for other livestock. The increase in fetal numbers seen in the grazing trial is consistent with previous studies. The level of response will vary depending on the difference in quantity and quality of lucerne and dead pasture.

However, the increased twinning rates achieved by grazing lucerne mean additional attention to pregnant ewe nutrition is required to maximise lamb survival, although the survival percentage was the same between treatments in this study.

Also avoid grazing lucerne with heavy aphid or fungal attack – in response lucerne produces higher levels of coumestrols which can reduce ovulation rates in ewes. **FA**

Avoid grazing lucerne with heavy aphid or fungal attack – in response lucerne produces higher levels of coumestrols which can reduce ovulation rates in ewes.

MORE LAMBS

Grazing lucerne rather than dead pasture resulted in an extra 19 lambs marked per 100 ewes joined. This equates to an increase in lambs marked per ewe from 96% to 115%.

There was no advantage or disadvantage in grazing lucerne past day seven of joining.

There was also no increase in the

seven days of joining can increase twinning rates and the number of lambs born.

Grazing lucerne for longer into joining did not reduce lambs born, but may not increase lamb numbers either if most ewes have mated during the first two weeks of joining.

This means for autumn joining, a 14-day lucerne grazing period is the most efficient, and after this, the ewes could be

Further reading:

To compare gross margin returns for flushing ewes on lucerne, refer to the Green Feed Tactical Management Calculator under 'tools' on the EverGraze website www.evergraze.com.au

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10.1.2 Appendix 2



March 2015

Animal Health Update

Joining ewes on Lucerne by District Veterinarian Amy Shergold

There are a few schools of thought about joining ewes on lucerne, many of which raise concerns about ewe fertility.

One theory suggests that high protein levels in lucerne increase blood urea, which leads to impaired fertility. High protein intake has been shown to have a detrimental effect on embryo development in sheep and cattle, but it is not clear that this would occur for females grazing lucerne. Recent unpublished trials funded by MLA and conducted at Charles Sturt University (CSU) failed to show a relationship between blood urea and foetal numbers for ewes eating lucerne.

Another idea is that lucerne contains a phyto-oestrogen, similar to the hormone oestrogen, which reduces ovulation rates. This is not commonly reported on lucerne. It becomes more of a risk if lucerne is infected with fungus or aphids as this increases phyto-oestrogen levels, so that if the infection is heavy, grazing during joining is best avoided.

The third hypothesis is that joining ewes on lucerne facilitates maximum feed intake, which causes a decrease in the hormone progesterone. This is supported by trial work done in the late 80s and early 90s, which shows days 11-12 after conception are the most critical for the adverse effects of low progesterone. This may explain farmer reports of poor results when joining ewes on lucerne in wet summers when pastures are lush (*pers comms* Graham, P).

Recent unpublished trials done at CSU (*pers comms* Robertson, S) have shown different results. Merino ewes were joined in March and were put on lucerne from 7 days before until the end of joining. Another group of similar ewes were taken off lucerne 7 days after the ram was put in, while a third group only grazed dead pasture. Grazing on lucerne produced 115% lambs marked per ewe compared with 96% if ewes grazed only dead pasture. This trial showed that staying on lucerne throughout joining did not reduce the number of lambs born. Taking ewes off lucerne at day 7 of joining was deemed most efficient as the pasture could be used for other purposes.

CSU trials were also done with artificially bred sheep. Ewes in this study were eating large quantities of lucerne for 17 days after insemination and had half the number of multiples when compared with ewes eating maintenance levels. It is unclear why this result was seen in artificially inseminated ewes and not naturally bred ewes.

The bottom line is that flushing naturally joined ewes on lucerne pasture can increase the number of lambs born. Recent trial work done by CSU demonstrated no problems with grazing ewes on large quantities of lucerne throughout the joining period in naturally bred sheep, but flagged a concern about above maintenance feeding in artificially bred sheep.

10.1.3 Appendix 3

Graham Centre website: www.grahamcentre.net

Ruminant Feedbase

Nutritional management to reduce embryo mortality in short-term flushed ewes

Primary Investigator	Dr Susan Robertson
Organisation	Charles Sturt University
Funding Source	MLA
Duration	2013-15
Location of Research	Wagga Wagga, NSW

Aim

Increasing the number of lambs weaned can increase the profitability of sheep production. Nutrition around joining has the potential to affect ovulation rate and embryo mortality, and so lambs born per ewe. Flushing on lucerne pasture has been recommended as a means of increasing the number of lambs born, but there has been concern in the sheep industry that grazing lucerne during joining causes embryo mortality.

MLA funded two studies which aimed to determine whether the quantity of lucerne consumed altered foetal numbers, and whether removal of ewes from lucerne during early pregnancy would increase or decrease foetal numbers.

Pen study

During 2013 and 2014, Merino ewes were oestrous synchronised, artificially inseminated (Day 0), and fed in group-pens one of 5 treatments:

1. Control – Fibre pellet Day -7 to 17 at maintenance
2. Freshly cut lucerne pasture at maintenance from Day 0 to 17
3. Freshly cut lucerne pasture ad libitum from Day 0 to 7
4. Freshly cut lucerne pasture ad libitum from Day 0 to 17
5. Freshly cut lucerne pasture ad libitum from Day -7 to 17



Pregnancy rates were similar in all treatments. However, feeding lucerne at ad libitum quantities for the first 17 days after insemination reduced the percentage of pregnant ewes with multiple foetuses (18%) compared with ewes fed at maintenance levels of lucerne (32%) or pellets (34%). The percentage with multiples was similar for ewes fed ad libitum for the first 7 or 17 days. The number of foetuses per pregnant ewe was reduced from 1.33 when fed at maintenance, to 1.21 when fed ad libitum.

It is recommended that artificially inseminated ewes should be fed at maintenance levels after insemination to minimise embryo mortality. Further research is needed to determine optimal feeding management for ewes.

Grazing study

A grazing study was conducted during 2014 to evaluate flushing with lucerne under commercial conditions with autumn-joined, naturally-mated ewes. Three treatments were compared:

1. Grazing dead pasture from Day -7 and throughout joining
2. Grazing live lucerne from Day -7 to Day 7 of joining, then dead pasture
3. Grazing live pasture from Day -7 and throughout joining



The rate of pregnancy was similar between treatments. The number of foetuses per ewe joined was increased by 30% for ewes grazing lucerne (lucerne 1.6 compared with dead pasture 1.3), which resulted in ewes grazing lucerne marking 115% lambs compared with 96% lambs from ewes joined on dead pasture. Leaving ewes on lucerne throughout joining produced the same number of lambs as removing ewes after day 7 of joining. Flushing ewes on lucerne was highly effective at increasing the number of lambs born and marked.

Recommendations

Artificially inseminated ewes are best fed at maintenance levels after insemination to maximise foetal numbers

Grazing naturally cycling ewes on leafy lucerne pasture for 7 days before joining and the first 7 days of an autumn joining can produce large increases in twinning rates and the number of lambs born. The level of response will vary with the quality and quantity of lucerne.

Naturally cycling ewes can remain on lucerne throughout joining without risking a reduction in foetal numbers, but there may be no increase in foetal numbers if most ewes mate and fall pregnant in the first 14 days of joining. This means that if there is a limited quantity of lucerne, ewes can be removed from lucerne at day 7 of joining while still gaining an increase in the number of foetuses.

Avoid grazing lucerne heavily infected with fungus or aphids – this is known to reduce ovulation rates in ewes.

Higher twinning rates result from flushing – producers need to appropriately manage the higher nutritional needs of twin-bearing ewes and twin-born lambs, and lamb survival, to gain the most benefit from flushing ewes.

10.1.4 Appendix 4.

Update EverGraze website Flushing key message (21 April 2015)

Embryo Mortality trial results to update EverGraze website

Putting the Research into practice

Timing of grazing

Remove the sentence 'CSU is undertaking....on embryo mortality'.

Replace with:

In 2013 and 2014 CSU conducted two studies, funded by Meat & Livestock Australia, to determine whether the timing and quantity of lucerne eaten by ewes would alter fetal numbers. Some parts of the sheep industry are concerned that grazing lucerne throughout joining could cause embryo mortality, reducing the number of lambs born. These studies tested whether access to lucerne only to day 7 or for 17 days (pen study) or throughout joining increased fetal numbers.

Pen study: artificially inseminated ewes were fed fresh lucerne or pellets in pens for 7 days before and 17 days after insemination.

The same number of fetuses were produced by ewes fed maintenance levels of pellets or lucerne. Feeding lucerne at *ad libitum* levels after insemination produced 12% less fetuses than if ewes were fed at maintenance levels. Pregnancy rates were not reduced, but the proportion of ewes with twin lambs was.

Grazing study: naturally cycling autumn-joined ewes were grazed on lucerne for 7 days before joining and to either day 7 of joining or throughout a 36 day joining. These were compared with ewes only grazing dead pasture.

Grazing on lucerne produced 30% more fetuses per ewe compared with only grazing dead pasture (1.6 compared with 1.3), resulting in 115% lambs marked rather than 96% per ewe joined. Leaving ewes on lucerne throughout joining did not reduce fetal numbers or pregnancy rates.

Grazing naturally cycling ewes on lucerne before and during joining is a means of increasing lambs born. If limited lucerne is available, ewes can be removed after 7 days while still getting most ewes flushed. However, leaving ewes grazing lucerne to the end of joining does not reduce lambs born. Artificially mated or fed ewes should only be fed at maintenance levels to avoid reductions in lambs born.

10.1.5 Appendix 5.

Proc. Aust. Soc. Anim. Prod. 2014 Vol. 50

Lucerne pasture *ad libitum* after day 7 post insemination may increase embryo mortality in ewes

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Embryo mortality was increased when ewes were fed at twice maintenance levels of energy (Parr 1992) or fed diets high in protein or urea (McEvoy *et al.* 1997). Grazing live lucerne pasture during the peri-conceptual period increases ovulation rate and fetal number where ewes are removed from pasture 7 days into the joining period (Robertson *et al.* 2013). It is not clear whether a longer period of grazing lucerne would increase fetal numbers due to more ewes being flushed, or reduce them due to increased embryo mortality. The aim of the current experiment was to determine whether the level and timing of feeding fresh lucerne pasture would effect fetal number and embryo mortality.

As part of a two year study, a group-fed pen experiment was conducted in 2013. Merino ewes (175) in mean condition score 3.1 ± 0.03 and weighing 55.6 ± 0.50 kg were allocated to one of five treatment diets (two replicates per treatment). Ewes were fed daily in troughs either maintenance levels of a faba and oat hull based pellet, or fresh lucerne pasture, or *ad libitum* levels of lucerne at different times between day -7 and 17 from artificial insemination. Between days -7 and 17, if not being fed lucerne, ewes were fed a maintenance ration of the pellet. An irrigated lucerne pasture was mown (finely chopped) daily with maintenance rations fed at 0.9 kg DM/ewe daily. Oestrous cycles were synchronised, 200 IU gonadotrophin used, and ewes artificially inseminated. Embryo losses were estimated as the difference between fetal numbers at day 55 of pregnancy, measured using trans-abdominal ultrasound, and the number of corpora lutea recorded by rectal ultrasound on day 10 post insemination. The sex of lambs was recorded at birth. Data was analysed using linear mixed models, with a binomial distribution for categorical data where proportions were assessed.

Mean ovulation rate per ewe, the number of fetuses per ewe, the proportion of ewes which were pregnant or bearing multiples, and estimated embryo mortality were not different ($P > 0.05$) between treatments (Table 1), probably due to low numbers of ewes. The number of fetuses per ewe was numerically (20%) lower when ewes were fed *ad libitum* levels of lucerne beyond day 7 after insemination compared with ewes fed a maintenance pellet. Feeding *ad libitum* levels of lucerne between days 0 and 7 after insemination resulted in similar ($P > 0.05$) embryo retention and proportion of ewes with multiples as the control, suggesting *ad libitum* levels of lucerne days 0-7 was not detrimental. Fewer ($P < 0.05$) female lambs were born to ewes fed lucerne before insemination, compared with after, and the role of fatty acids in this is being evaluated. While studies continue, any risk of increased embryo loss is avoided by removing ewes from high levels of lucerne pasture after joining.

Table 1. Reproductive performance of ewes fed different diets in the days around insemination (day 0)

Feed	Treatment	No. fetus per ewe	Proportion pregnant ewes	Proportion with multiple fetuses	Proportion pregnant ewes with multiple fetuses	Proportion of ewes with total embryo retention	Proportion female lambs
Pellet	Control maintenance	1.00	0.74	0.26	0.36	0.50	0.38 ab
Lucerne	0-17 maintenance	0.94	0.67	0.27	0.41	0.55	0.48 b
Lucerne	0-17 <i>ad libitum</i>	0.84	0.69	0.19	0.23	0.38	0.52 b
Lucerne	-7-17 <i>ad libitum</i>	0.79	0.64	0.15	0.24	0.33	0.17 a
Lucerne	0-7 <i>ad libitum</i>	0.94	0.71	0.24	0.33	0.47	0.48 b
	Mean sem	0.13	0.08	0.07	0.10	0.09	

a,b: Different letters within column indicate means differ at $P < 0.05$.

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10.1.6 Appendix 6.

Paper accepted for publication in the scientific journal Animal Reproduction Science on 23 April 2015, subject to provision of high resolution graphics files.

Reproductive performance in ewes fed varying levels of cut lucerne pasture around conception

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ABSTRACT

Elevated intakes of protein and energy may increase embryo mortality, but it is not clear whether fresh lucerne (*Medicago sativa*) pasture poses a risk. A two-year pen study using oestrous synchronised and artificially inseminated Merino ewes (n = 175 in 2013 and 215 in 2014) evaluated whether feeding freshly cut lucerne pasture (mean crude protein 19.7%, metabolisable energy 9.4 MJ/kg DM) at maintenance or *ad libitum* during different periods around insemination altered reproductive performance in comparison with ewes fed a Control diet (mean crude protein 7.8%, metabolisable energy 9.0 MJ/kg DM) of pelleted faba bean hulls and oat grain hulls at maintenance. The proportion of pregnant ewes carrying multiple fetuses was reduced (P=0.026) when ewes were fed lucerne *ad libitum* between days 0 and 17 after insemination compared with the Control diet (0.18 and 0.34, respectively), but not when ewes were fed lucerne *ad libitum* between days 0 and 7 after insemination (0.22). Reproductive performance, including the proportion of ewes pregnant and the proportion with multiple fetuses, was not different (P>0.05) when ewes were fed lucerne at maintenance between days 0 and 7 compared with the Control diet. While reproductive performance was similar when ewes were fed lucerne at maintenance between 0 and 17 days after artificial insemination compared with pellets at maintenance, fetal numbers per pregnant ewe were reduced by feeding lucerne *ad libitum* after insemination.

Keywords: sheep, nutrition, flushing, embryo survival

1. Introduction

Peri-conceptual nutritional management which enhances both ovulation rate and embryo survival is required to increase the number of lambs born. Reproductive potential is limited by ovulation rate, but failure in fertilisation, embryo, fetal and lamb survival all contribute to how much of the potential is attained, with failure of multiple ovulations to result in fetuses an important source of inefficiency in commercial flocks (Kleemann and Walker 2005).

Ovulation rates can be elevated by increasing the energy or protein intake of ewes (Knight *et al.* 1975; Nottle *et al.* 1990; Smith and Stewart 1990; Vinales *et al.* 2005), which has been achieved using corn/soybean grain (Vinales *et al.* 2009) lupin grain (Nottle *et al.* 1997) and live pastures (King *et al.* 2010). While responses in ovulation rate may be achieved most efficiently through short-term increased nutrition between approximately days 10 to 14 of the oestrous cycle (Stewart and Oldham 1986), this targeted duration of feeding can only accurately be achieved if oestrus is synchronised. In commercial flocks where oestrus is not synchronised, extending the duration of increased nutrition into early pregnancy for some ewes is required in order for a large proportion of ewes to receive elevated nutrition pre-ovulation.

While ovulation rate can be increased by feeding increased energy or protein prior to ovulation, a high intake of energy or protein during early pregnancy may also increase embryo mortality (Parr *et al.* 1987), which may prevent a net improvement in reproductive performance. Feeding at twice maintenance levels has been shown to cause embryo mortality in both naturally cycling (Cumming *et al.* 1975) and oestrous synchronised (Parr *et al.* 1987) ewes, compared with ewes fed at maintenance levels. The clearance rate of progesterone through the liver is higher when feed intake is increased, which leads to reduced circulating concentrations of progesterone (Parr *et al.* 1993) that may be associated with lower pregnancy rates (Parr *et al.* 1987). The sheep embryo appears to be most sensitive to reduced progesterone levels on days 11 and 12 post mating (Parr 1992), although the duration of time for which progesterone is reduced influences the extent to which pregnancy rates are reduced (Ashworth *et al.* 1987). Embryo mortality may also be increased when ewes consume high levels of dietary protein or nitrogen around conception (McEvoy *et al.* 1997; Bishonga *et al.* 2006; Meza-Herrera *et al.* 2010). This effect has particular relevance where flushing with high protein lupin grain or lucerne pasture is being advocated and some recommendations indicate that ruminants should not graze high nitrogen pastures around mating (Robinson *et al.* 2006). However, high numbers of fetuses per ewe (1.5) have been achieved by flushing Merino ewes on lucerne pasture containing over 21% crude protein (Robertson *et al.* 2014b).

The mean number of fetuses per ewe joined was higher when unsynchronised ewes grazed lucerne compared with cereal stubble *ad libitum* for 7 days prior to and only for the first 7 days of joining, with ewes removed at this time in order to avoid high energy and protein intakes during day 11-12 post conception when the embryo is most at risk of loss (Robertson *et al.* 2014b). Removing ewes from lucerne at day 7 of joining, however, limits the proportion of ewes which mate at a flushed ovulation to approximately 65%, potentially restricting the number of lambs born. The effect of feeding lucerne for a longer period of time to increase the proportion of ewes fed pre-mating on subsequent embryo mortality is currently unknown. Therefore, the aim of the current study was to determine whether the

quantity of fresh lucerne pasture fed, and the timing of feeding during the peri-conceptual period, influenced estimated embryo mortality and the number of fetuses per ewe.

2. Materials and methods

2.1. Experimental design

The study was approved by the Charles Sturt University Animal Care and Ethics committee (project no. 21/105). The experiment was conducted during 2013 and 2014.

The pen-feeding experiment evaluated five treatments in a randomised block design with two replicates of each treatment. Where day 0 = day of artificial insemination and maintenance (M) = energy requirement for maintenance, the treatments comprised:

6. Control – Fibre pellet Day -7 to 17 at maintenance
7. Freshly cut lucerne pasture at maintenance from Day 0 to 17
8. Freshly cut lucerne pasture *ad libitum* from Day 0 to 7
9. Freshly cut lucerne pasture *ad libitum* from Day 0 to 17
10. Freshly cut lucerne pasture *ad libitum* from Day -7 to 17

When not being fed lucerne, ewes were fed the maintenance (Control) pellet for the duration of pen feeding (23 January to 3 March 2013 and 16 January to 25 February 2014, Days -22 to 17). The lucerne cultivars used were Pioneer L56 in 2013, and WL925HQ in 2014. The irrigated lucerne pasture was cut daily with a commercial lawnmower (Iseki SF370 outfront; www.iseki.co.jp) to provide fresh lucerne, and the chop size prevented selection of lucerne components. A low protein and low grain commercial maintenance pellet based on faba bean hulls and oat grain hulls was purchased from a commercial supplier (Fibre Pellet; Conqueror Milling Company, Cootamundra, NSW). The ingredients of the pellet are commercial in-confidence, however the manufacturer stated that the pellet did not contain whole grain or urea.

Ten outdoor group-feeding pens, each measuring 6 x 8 m and containing feed and water troughs and shade, were randomly allocated to treatment and replicate. In 2013, on Day -22, two weeks prior to the commencement of differential feeding, a mixed age flock of 176 Merino ewes were stratified on age and randomly allocated to the pens based on condition score and live weight. In 2013 replicate 1 and 2 contained 18 or 17 ewes per pen, respectively. In 2014, the same ewes were stratified on pen number and pregnancy status in 2013, then current condition score and liveweight. Half the ewes remained in the same treatment as in 2013, with the remainder being randomly allocated to the other treatments. An additional five or six 18 month old ewes were randomly allocated to each pen, totalling 22 or 21 ewes per pen in replicates 1 and 2, respectively.

2.2. Sheep management

The ewes were vaccinated (1 ml Glanvac 6 in 1; Pfizer Animal Health, West Ryde, Australia) as they entered the pens. Controlled internal release devices (Eazi-breed CIDR sheep and goat; Pfizer Animal Health, a division of Pfizer Australia Pty Ltd, West Ryde, Australia)

containing 300mg progesterone were inserted on Day -16, and removed on Day -2. At CIDR removal the ewes were injected with 1ml (200iu) Pregnenol serum gonadotrophin (Bioniche Animal Health (A/Asia) Pty Ltd, Armidale, Australia). Fresh semen from Merino rams was used to artificially inseminate the ewes via laparoscopy on Day 0, after a 12 h fast. Semen from each ram was distributed evenly between treatment groups to avoid potential ram bias. The number of corpora lutea was measured using rectal ultrasound on Days 8 or 9 after insemination (Vinoles *et al.* 2004) using an Ibex Pro, Portable Ultrasound System with a 5.0 MHz Sector probe (E. I. Medical, Loveland CO, USA). The ewes were removed from the pens on Day 18 and grazed as one flock. Rams were introduced to the ewes on Day 29 in 2013 and removed on Day 50 2013, but only data resulting from the artificial insemination was analysed. The number of fetuses was determined by trans-abdominal ultrasound scanning by a commercial operator 57 days (2013) or 60 days (2014) following artificial insemination.

One week prior to the due lambing date ewes were vaccinated (1 ml Glanvac 6 in 1; Pfizer Animal Health, West Ryde, Australia). Ewes pregnant to the insemination or natural mating were separated and placed in different lambing paddocks. During lambing lambs were identified to their mothers, tagged and sex and birth weight recorded. Dead lambs were recorded and removed from the paddock. The presence of lambs at marking (20 September 2013 or 12 August 2014) was used to calculate lamb survival.

Ewe weight after overnight fasting was recorded at allocation (Day -22) and when ewes were removed from pens (Day 18). Unfasted weights were recorded prior to feed treatments (Day -8), on Day 8, and one week prior to the start of lambing. Ewe condition score (scale 0 (emaciated) to 5 (obese)) (Jefferies 1961) was recorded at the same times, except on Day 8.

2.3. Feeding

The quantity of pellet fed was adjusted prior to Day -8 to achieve maintenance of liveweight. The daily ration fed after Day -8 was 850 g DM/ewe or 1.19 kg DM/ewe in 2013 and 2014, respectively. Ewes fed the maintenance diet of lucerne were fed 3.6 kg fresh lucerne/day in 2013. In 2014 the ewes were initially fed 4.5 kg fresh weight, but because the ewes were losing weight, the quantity was increased to 5.0 kg/ewe on Day 13. *Ad libitum* quantities of lucerne were fed at approximately twice these levels, with quantities adjusted daily to ensure refusals were obtained.

The rations were fed once daily at 10 am so that blood sampling could occur approximately 2 hours after feeding. The exception was on Day 0 when ewes were fed in the afternoon following artificial insemination. Feed refusals were collected daily, and dry matter determined to allow calculation of feed intake. Samples of fresh cut lucerne were also collected daily, weighed, then dried at 60°C to constant weight for calculation of dry matter and retained for measurement of nutritive value.

A mineral lick block containing no urea (2013 - Olssons 12% high Sulphur with trace elements; 2014 - Olssons New England; Olssons, Yennora New South Wales) was placed in each pen for the duration of pen-feeding and were replaced as consumed. The ewes consumed 0.030 ± 0.0018 kg daily in 2014, with no differences ($P > 0.05$) between treatments; the quantity consumed was not recorded for 2013.

2.4. Blood collection and analyses

On Day -8 (2013) or -9 (2014) (before differential feeding), and Days 5, 12 and 17, blood samples were collected from the jugular vein of eight randomly selected ewes per pen into 2 x 9ml lithium heparin vacutainers. Samples were collected from the same ewes on each occasion within each year, commencing at 12 noon each time. Samples were stored on ice then centrifuged at 1500 x g for 10 minutes, and plasma and red cell samples separated within 5 hours of collection, and stored at -20 °C until analysis for urea, progesterone and leptin. Urea was analysed using an Infinity™ Urea Liquid Stable Reagent (Fisher Diagnostics, Middletown, USA) in 2013, and in 2014 using an automated enzymatic method via urease and glutamate dehydrogenase (Randox Laboratories Ltd. Crumlin, UK, product # UR 221) (Tiffany *et al.* 1972). Progesterone was measured in duplicate using an Immunotech RIA Progesterone kit IM1188 (Beckman Coulter Gladesville, NSW 2111, Australia). Leptin was analysed by radioimmunoassay (Blache *et al.* 2000). Ammonia was analysed using an enzymatic method with a commercial reagent (Thermo Trace DST Liquid Reagent).

2.5. Feed analyses

Samples of the pellets were obtained for nutrient analysis. Daily samples of lucerne were bulked over approximately weekly periods within years prior to analysis. Proximate analyses (% DM) were determined using near infra-red reflectance (NIR) spectroscopy with a Bruker multi-purpose analyser (MPA, Bruker Optik GmbH, Ettlingen, Germany) and OPUS software (version 5.1) using calibrations developed by the NSW Department of Primary Industries Feed Quality Service (NSW DPI FQS, Wagga Wagga, New South Wales) as described previously (Packer *et al.* 2011). Proximate analyses of the Control pellet were determined by wet-chemistry by the same methods used for NIR calculations except that total lipid concentration was determined by solvent extraction using the Randall method (Randall 1974) with hexane which has been validated for use with animal feed (Thiex *et al.* 2003) using a FOSS solvent extraction system (Soxtec 2050 Avanti Extraction Unit, FOSS, Hoganas, Sweden). Crude protein (CP) was estimated from nitrogen (N, CP (% DM) = N (% DM x 6.25)) and metabolisable energy (ME) was estimated from digestible organic matter in the dry matter (DOMD, lucerne ME (MJ/kg DM) = 0.203 DOMD (%) – 3.001; commercial pellet ME (MJ/kg DM) = 0.138 x DOMD (%) + 0.272 x Ether Extract (%) + 0.858 (Anon. 2007)).

2.6. Statistical analyses

Data were analysed using Genstat® 16th edition (VSN International 2013). Condition score data was analysed using linear mixed modelling with repeated measurements, using condition score at allocation as a co-variate, treatment and year as the fixed effects and pen within year as the random effect. Unfasted live weight data at the start of differential feeding (Day -8) and fasted weight when ewes were removed from pens were analysed separately using linear mixed models with weight at allocation as a co-variate, treatment and year as fixed and pen within year as the random effect. Linear mixed models were also used to analyse the number of corpus lutea and fetuses per ewe. Embryo retention or loss was estimated for each ewe from the difference between the number of corpora lutea and fetal

number. Generalised linear mixed modelling using a binomial distribution was used to analyse lamb survival, embryo losses and the proportion of ewes with multiple ovulations and fetuses, with the logit transformation meaning standard errors for backtransformed means were not available. Treatment and year were used as the fixed effects and pen within year as the random effect. Birth type was also included as a fixed effect for lamb survival. Main effects (fed maintenance only or *ad libitum* lucerne for any time) were analysed separately.

Progesterone (transformed by natural logarithm), urea and leptin concentrations were analysed using linear mixed models with concentration at Day -8 as a co-variate, with pregnancy status, treatment, day of sampling and year as the fixed effects and tag, pen and year as random effects. Linear regression was used to evaluate associations between either pregnancy status or fetal number and progesterone or urea at each day of sampling. Generalised regression using a binomial distribution was also used to evaluate the association between urea and proportion of ewe with multiple fetuses and partial and total embryo loss on Day 5, 12 and 17. Non-significant ($P>0.05$) terms and interactions were removed from the models. An alpha of 0.05 was used for all statistical tests.

3. Results

3.1. Feed intake

The estimated nutritive content of feeds is shown in Table 1 and the estimated mean daily energy and protein intake of ewes during pen-feeding is shown in Table 2. Intake at maintenance levels tended to be higher in 2014 than in 2013. Compared with ewes fed the Control pellet, the energy intake of ewes fed a maintenance ration of lucerne varied between -15 and + 17%, while protein intake was two to five times higher. The energy intake of ewes fed *ad libitum* lucerne between Days 1 and 17 was 175 to 200% that of the control in 2013, but only 132 to 160% of the control in 2014. Heavy rainfall overnight on Day 7 and 8 resulted in high levels of lucerne refusals between Days 8 and 11 in 2014, although between Days 12 and 17, intake increased to between 140 and 260% that of the Control ewes.

Insert Table 1 here

Insert Table 2 here

3.2. Condition score and live weight

The ewes were in moderate condition score at allocation (Day -22) in both years (3.1 ± 0.03 and 2.7 ± 0.02 , for 2013 and 2014, respectively). The condition score of Control ewes was similar to that of ewes in all other treatments at the start of differential feeding on Day -8.

The mean fasted weight of ewes at allocation (Day -22) was higher ($P<0.05$) in 2013 (55.6 ± 0.50) than in 2014 (49.9 ± 0.46). The mean unfasted weight of ewes at the start of differential feeding (Day -8), using the weight at allocation as a co-variate, was similar between treatments in both years, but the mean fasted weight at Day 18 was higher in ewes fed *ad libitum* levels of lucerne to Day 17 than for ewes in all other treatments (Fig. 1). The weight of ewes fed maintenance lucerne was similar to Control ewes at Day 18 in 2014, but 1.7 kg lighter ($P<0.05$) in 2013. The mean weight change between fasted weights at allocation and Day 18 differed ($P<0.05$) between years because ewes in all treatments lost

between 3.5 and 7.6 kg in 2013, but in 2014 ewes in groups fed *ad libitum* lucerne to Day 17 gained at least 3.9 kg, while ewes in all other treatments lost between 0.4 to 2.6 kg.

Insert Fig. 1 here

3.3. Embryo mortality and fetal numbers

Similar numbers of ewes were pregnant between treatments after backup mating to rams in 2013, such that the lambing history of ewes for that year was similar. For 2013 and 2014 reproductive data resulting from artificial insemination, the interactions between treatment and year were not significant. No triplet pregnancies were identified. The mean proportion of ewes with multiple ovulations was not higher ($P=0.07$) when ewes were fed lucerne *ad libitum* for seven days prior to insemination and to Day 17 (0.83), compared with the Control (0.65) (Table 3).

The proportion of ewes pregnant was not reduced when ewes were fed lucerne compared with the Control diet. However, the proportion of pregnant ewes with multiple fetuses was reduced ($P=0.026$) by feeding lucerne *ad libitum* from Day 0 to 17 after insemination, compared with feeding either pellets or lucerne at a maintenance level (Table 3). The proportion of pregnant ewes with multiple fetuses was not reduced by feeding lucerne *ad libitum* between days 0 and 7 or -7 and 17 compared with the Control. When the main effect of level of feeding was considered, the proportion of pregnant ewes with multiple fetuses was reduced ($P=0.001$) by one third, when ewes were fed *ad libitum* compared with at maintenance. The number of fetuses per ewe was not reduced when ewes consumed lucerne at any level compared with the Control diet, but the standard errors were large. Fetal number per pregnant ewe was reduced ($P=0.046$) by 0.12 fetuses/ewe by the main effect of feeding *ad libitum* lucerne compared to maintenance feeding.

Insert Table 3 here

The proportion of ewes assumed to retain all embryos (fetal number the same as number of corpora lutea) was lower ($P=0.031$) for the treatment fed lucerne *ad libitum* Days -7 to 17, in comparison with those fed either lucerne or pellets at maintenance levels after insemination (Table 3). The partial loss of embryos was not significantly ($P=0.095$) higher when ewes were fed lucerne *ad libitum* for longer than seven days post-insemination, however, the main effect of level of feeding was significant and the partial loss of embryos was greater when ewes were fed *ad libitum* compared with at maintenance (Table 3).

3.4. Lambing data

The mean duration of gestation was 151 ± 0.14 days, with no differences ($P=0.648$) between treatments. The number of lambs born per pregnant ewe, the survival of lambs from birth to marking, and the number of lambs marked per ewe inseminated or per pregnant ewe were similar between treatments (Table 4). The number of lambs born per pregnant ewe tended to be greater ($P = 0.086$) for ewes fed at maintenance compared to if fed *ad libitum* (1.31 ± 0.047 cf. 1.21 ± 0.040 , respectively) when analysed as main effects. The survival of multiple born lambs was similar ($P=0.170$) to that of singles (proportions 0.69 and 0.77, respectively). The birthweight of lambs was similar between treatments, although twin lambs were lighter ($P<0.05$) than single-born lambs (4.1 ± 0.09 cf. 5.2 ± 0.06 kg, respectively).

Insert Table 4 here

3.5. Blood parameters

Plasma urea concentrations at Days 5, 12 and 17 were not influenced by the pregnancy status of ewes. Urea concentrations were always lower ($P < 0.05$) for ewes when fed pellets than when fed lucerne, and remained below 4 mmol/L, but ewes fed maintenance levels of lucerne did not consistently have lower urea than those fed lucerne *ad libitum* (Fig. 2). The urea concentration for ewes fed lucerne was higher ($P < 0.05$) in 2014 than in 2013, and for groups fed *ad libitum* lucerne, plasma urea remained below 9 mmol/L in 2013, but at or above this level in 2014. Fetal number per ewe was not ($P > 0.05$) associated with urea concentration on any of the days sampled. The proportion of ewes with multiples, partial and total embryo loss were also not associated with urea concentration on Days 5, 12 or 17.

Insert Fig. 2 here

Plasma progesterone concentrations at Day 17 were lower ($P = 0.01$) in non-pregnant compared with pregnant ewes in both years. At Days 5 and 12, progesterone was only lower ($P < 0.05$) in non-pregnant compared with pregnant ewes in 2013, not in 2014.

The concentration of plasma progesterone was not different between dietary treatment groups when ewes were pregnant at any day of sampling (Fig. 3). For non-pregnant ewes, however, the concentration of progesterone was significantly lower ($P = 0.006$) 17 days after artificial insemination when ewes were fed lucerne at any level compared with the Control diet (Fig. 3), and lower at Day 12 when ewes were fed lucerne from Day -7 compared with Control ewes.

Insert Fig. 3 here

3.6. Relationship between progesterone and pregnancy outcome

The change in progesterone between Days -8 and 5 was similar ($P > 0.05$) between pregnant and non-pregnant ewes (-1.92 ± 0.219 and -1.63 ± 0.169 ng/ml, respectively) and treatments. The increase in progesterone concentrations between Days 5 and 12 was larger ($P = 0.02$) in pregnant ewes (1.88 ± 0.13 ng/ml) compared to non-pregnant ewes (1.40 ± 0.166 ng/ml), with no interaction with treatment. The increase in progesterone between Days 5 and 12 was less ($P = 0.03$) for ewes which were fed *ad libitum* levels of lucerne to Day 17 (up to 1.29 ± 0.248 ng/ml), compared with those in the Control treatment (2.11 ± 0.249 ng/ml).

Fetal number per ewe increased with progesterone concentration at both Day 5 ($y = 0.3372 + 0.2216x$; $r^2 = 0.15$; $P < 0.001$) and Day 12 ($y = 0.238 + 0.1472x$; $r^2 = 0.16$; $P < 0.001$). Although significant ($P < 0.05$), less of the variation (6%) in fetal numbers was explained by the change in progesterone between these days. The proportion of ewes pregnant was lower ($P < 0.001$) in ewes with progesterone levels 1 ng/ml or less at Day 5 after insemination, compared with those with 1.5 to 2 ng/ml or higher (Fig 4). At Day 12, the proportion of ewes pregnant was lower ($P < 0.001$) for ewes with progesterone of 2 ng/ml or less (0.30 ± 0.096), in comparison with ewes with 2 to 3 ng/ml (0.62 ± 0.072) or more.

Insert Fig. 4 here

3.7. Leptin

Plasma leptin concentrations were higher ($P < 0.05$) in 2014 than in 2013, were higher ($P < 0.05$) in pregnant compared with non-pregnant ewes, and were lower ($P < 0.05$) in ewes fed maintenance levels of lucerne (0.94 ± 0.042 ng/ml) than in all other treatments ($\geq 1.08 \pm 0.044$ ng/ml). While leptin concentrations were similar between pregnant and non-pregnant ewes at Days 5 and 12 after insemination, they were higher ($P < 0.05$) in pregnant ewes at Day 17 (Fig. 5). The interactions between treatment and pregnancy status or day of sampling were not significant.

Insert Fig. 5 here

4. Discussion

The proportion of pregnant ewes with multiple fetuses was nearly halved by feeding *ad libitum* quantities of freshly cut lucerne pasture to ewes from insemination for 17 days, compared with maintenance quantities of lucerne or the control pellet over the same period. The reduction in multiple fetuses may have been due to either embryo mortality or fertilisation failure since the estimated ovulation rates of ewes in these treatments was similar, although the mechanism cannot be determined from the current study. The proportion of ewes with multiple fetuses was not significantly lower when ewes were fed lucerne *ad libitum* to Day 7 post-insemination compared with those fed the Control and lucerne *ad libitum* to Day 17 diets, although it was numerically closer to the latter. It is likely that there were insufficient numbers of ewes to achieve significance in these comparisons, and the large numerical difference compared to maintenance feeding suggests caution in feeding *ad libitum* levels of lucerne post-insemination, even if *ad libitum* feeding ceases after only seven days. The proportion of ewes with multiple fetuses was also not reduced by feeding lucerne from Day -7 to 17 in comparison to the control diet, even though it was markedly lower. The proportion of ewes with multiple ovulations differed numerically by 18%, and this impact of pre-insemination feeding of lucerne was not dissimilar to that expected through comparison with previous studies (King *et al.* 2010). The 18% difference was probably not significantly different due to insufficient numbers of ewes. However, a similar number of fetuses per ewe resulted from the higher ovulation rate, indicating higher reproductive failure for ewes fed lucerne *ad libitum* from Day -7 to 17 compared with a maintenance diet. Ideally, an additional treatment of ewes fed lucerne at *ad libitum* levels from Day -7 to Day 0 would have been included in the experiment to isolate the effect of flushing from post-insemination nutrition, but this was not logistically possible.

The conclusion that feeding *ad libitum* levels of lucerne in early pregnancy may have increased embryo mortality is consistent with previous studies where ewes were fed at twice maintenance (Cumming *et al.* 1975; Parr *et al.* 1987), although a high rate of feeding has not increased embryo mortality in other studies (Lightfoot *et al.* 1976). Pregnancy rates were not reduced in the current study, in contrast to that of Parr (1987). A possible explanation for this difference is that a reduced pregnancy rate results from a larger severity or duration of challenge, whereas a less severe challenge may only result in partial loss of multiple embryos, expressed as a reduced rate of multiple fetuses. There is evidence for this process in the study of Ashworth *et al.* (1987), where low progesterone levels induced only on day 9 of pregnancy did not reduce pregnancy rates, although the proportion of ewes

retaining both twin embryos was halved, yet low progesterone induced on days 9, 10 and 11 of pregnancy resulted in 18% of ewes pregnant compared with 85% of control ewes.

It is possible the rate of multiple pregnancies was reduced by feeding lucerne *ad libitum* via alteration of progesterone concentrations. Progesterone levels and the rate of increase in progesterone between Days 5 and 12 were reduced by *ad libitum* feeding of lucerne. Pregnancy rate and fetal number were also positively associated with progesterone concentration. Increased feed intake is known to reduce circulating (jugular) progesterone levels, with progesterone declining within 2 hrs of a discrete feed and not returning to pre-feeding levels for 8 hrs (Parr *et al.* 1993). Clear associations between circulating progesterone levels and embryo mortality or pregnancy rate have previously been shown in sheep (Parr *et al.* 1987; Ashworth *et al.* 1989), and the mechanisms by which low progesterone concentrations both prior to and post ovulation may alter embryo survival have been reviewed (Morris and Diskin 2008; Diskin *et al.* 2012). Progesterone concentrations are influenced by the number of corpus lutea (Wilkins 1989) and embryo survival is reduced at high ovulation rates (Ashworth *et al.* 1989). In our study, the estimated number of corpus lutea was similar between treatments with the exception of ewes fed *ad libitum* lucerne prior to insemination, therefore differences in ovulation rate influencing progesterone do not explain the reproductive failure in the 0-17 lucerne *ad libitum* treatment.

The associations between progesterone levels at both Day 5 and 12 after insemination and reproductive failure are consistent with literature. While low progesterone levels are required post-ovulation, progesterone levels need to increase from day 4 or 5 to maintain pregnancy in ewes (Wilmot *et al.* 1985). In addition, the rate of increase in progesterone has previously been associated with embryo survival in sheep (Ashworth *et al.* 1989) and cattle (Kenyon *et al.* 2013). Both the level of progesterone at various time points, and the rate of increase during early pregnancy are quadratically related to embryo survival (Ashworth *et al.* 1989; Diskin *et al.* 2012), such that both insufficiency and excess lead to suboptimal outcomes. The observation in the current study that pregnancy rates were lower for ewes with progesterone levels of 2 ng/ml or less at day 12 after insemination is consistent with previous studies (Parr *et al.* 1987).

Fetal numbers were not associated with plasma urea concentrations. Embryo survival was similarly unrelated to plasma urea concentrations in beef cattle gaining weight while grazing nitrogen fertilised pasture containing 23% crude protein (Kenny *et al.* 2001), comparable to the protein levels of lucerne. These results are consistent with the conclusions of Velazquez (2011) that high plasma urea levels will not adversely impact embryo survival where ruminants are not in large negative energy balance, and the nitrogen levels fed are not extreme.

Feed type as such is unlikely to have caused the adverse effect of feeding *ad libitum* lucerne since pregnancy rates and fetal numbers were similar for treatments fed maintenance quantities of lucerne or pellets. This is supported by evidence that grazing of lucerne pasture is known to produce similar fetal numbers as grazing some other live pastures (Robertson *et al.* 2014b). Ovulation rates can be reduced by consumption of lucerne (Scales *et al.* 1977) where aphid or fungal attack has increased coumestrol levels in lucerne plants (Smith *et al.* 1979), but this can be avoided by not grazing affected pastures. The limited evidence that grazing lucerne containing low coumestrol levels may increase embryo mortality (Smith *et al.* 1979) can be discounted since in that study it did not occur in all lucerne treatments and

rams were not rotated between treatments which may have contributed to the observed difference.

The current study provides evidence that feed intake at levels below twice maintenance can produce an adverse effect on the proportion of ewes with multiple fetuses. The estimated energy intake of ewes fed *ad libitum* to day 17 was only 132 to 160% of maintenance requirements in 2014. Other studies which have fed 1.5 x maintenance only fed to day 8 of pregnancy (Abecia *et al.* 1997), with no effect on pregnancy rate. Another study (Lightfoot *et al.* 1976) also produced no indication of lower conception or twinning rates where ewes were supplemented with 740 g lupin grain/day before and for the first 18 days of joining, estimated to provide above maintenance energy.

Peri-conceptual nutrition is known to alter some aspects of the physiology and behaviour of the progeny (for reviews see Ashworth *et al.* (2009) and Fleming *et al.* (2012)). The survival of lambs to marking was similar for the different feeding treatments despite differences in the proportion of multiple births. The survival of multiples can be expected to be lower than that of single-born lambs, although preferential management of ewes can remove this difference (Robertson *et al.* 2011), and that the survival of multiples was not lower in the current study may be due to insufficient numbers. However, evidence from the current study that the feeding strategies used were not detrimental to lamb survival is commercially important.

The sex ratio of lambs may also be of commercial importance. The effect of the dietary treatments on the sex of lambs born, and the potential involvement of dietary differences in fatty acids for determining sex ratio (Gulliver *et al.* 2013) will be evaluated in a separate paper.

The ovulation data, and the embryo loss calculated from it, should be interpreted cautiously because technical difficulty with measurement resulted in no records of ewes without corpora lutea, suggesting that both corpora lutea and follicles may have been counted. However, this does not alter the conclusions, since the fetal numbers were similar to lambs born per ewe, indicating fetal numbers were a reliable measure, and the pattern of estimated total embryo retention and partial loss are consistent with the differences in fetal numbers. The small differences in fetal numbers and lambs born per ewe indicates fetal losses after day 60 of pregnancy were small, consistent with other studies which show most losses occur before day 30 (Viñoles *et al.* 2012).

Conclusion

The results of the current study demonstrated that the proportion of ewes with multiple fetuses was reduced by feeding *ad libitum* quantities of freshly cut lucerne to synchronised and artificially inseminated ewes for 17 days after insemination, compared with maintenance feeding. The proportion of ewes with multiple fetuses was not reduced by feeding lucerne *ad libitum* for only 7 days after insemination, but the rate of multiples was similar to that from ewes fed for 17 days, so caution appears warranted when feeding lucerne post-insemination, and feeding maintenance levels of energy post-insemination is recommended.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Table 1 Mean nutritive value of pellets and lucerne pasture (\pm sem) on a dry matter (DM) basis in 2013 and 2014.

	2013		2014	
	Fibre pellet	Lucerne pasture	Fibre pellet	Lucerne pasture
Dry matter (%)	95	25 \pm 0.2	95	20 \pm 0.1
Neutral detergent fibre (% of DM)	65	51 \pm 1.4	50	47 \pm 2.0
Acid detergent fibre (% of DM)	40	34 \pm 0.9	27	32 \pm 1.0
Crude protein (% of DM)	6.7	18.2 \pm 0.43	8.8	21.2 \pm 2.32
Dry matter digestibility (%)	57	62 \pm 0.9	54	66 \pm 2.9
Digestible organic matter in the dry matter (%)	56	60 \pm 0.8	54	63 \pm 2.3
Metabolisable energy (MJ/kg DM)	9.1	9.1 \pm 0.16	8.8	9.7 \pm 0.46
Crude Fat (% of DM)	1.8	-	1.6	-

Table 2 Estimated mean (\pm sem) daily energy (MJ ME) and crude protein (g) intake of ewes during pen-feeding 2013 and 2014. Intakes on the day of insemination (Day 0) were reduced due to surgery and are not included.

Year	Days of Experiment	Control	0 to 17 M	0 to 7 <i>ad libitum</i>	0 to 17 <i>ad libitum</i>	-7 to 17 <i>ad libitum</i>
<i>Energy intake (MJ ME/day)</i>						
2013	-7 to -1	7.8 \pm 0.0	7.8 \pm 0.0	7.8 \pm 0	7.8 \pm 0	10.9 \pm 0.47
	1 to 7	7.8 \pm 0.0	9.1 \pm 0.20	13.7 \pm 0.50	14.2 \pm 0.54	15.6 \pm 0.57
	8 to 17	7.8 \pm 0.0	7.3 \pm 0.30	7.8 \pm 0	14.1 \pm 0.61	15.4 \pm 0.79
2014	-7 to -1	10.5 \pm 0.0	10.5 \pm 0.0	10.5 \pm 0	10.5 \pm 0	12.4 \pm 0.98
	1 to 7	10.5 \pm 0.0	8.9 \pm 0.52	14.6 \pm 0.64	13.9 \pm 1.03	16.8 \pm 0.94
	8 to 17	10.5 \pm 0.0	8.9 \pm 0.52	10.5 \pm 0	15.4 \pm 1.45	15.5 \pm 1.40
<i>Protein intake (g/day)</i>						
2013	-7 to -1	58 \pm 0.0	58 \pm 0.0	58 \pm 0.0	58 \pm 0.0	221 \pm 9.6
	1 to 7	58 \pm 0.0	174 \pm 4.0	259 \pm 9.4	271 \pm 9.9	294 \pm 10.8
	8 to 17	58 \pm 0.0	147 \pm 6.3	58 \pm 0.0	283 \pm 13.2	315 \pm 15.0
2014	-7 to -1	105 \pm 0.0	105 \pm 0.0	102 \pm 0.0	105 \pm 0.0	251 \pm 17.7
	1 to 7	105 \pm 0.0	188 \pm 9.5	354 \pm 15.5	291 \pm 25.3	410 \pm 23.0
	8 to 17	105 \pm 0.0	208 \pm 13.1	130 \pm 0.0	361 \pm 34.2	334 \pm 28.7

Table 3 Mean reproduction outcomes and estimated embryo loss for ewes fed a control diet or lucerne fed at maintenance or *ad libitum* around the time of artificial insemination (2013 and 2014).

Reproduction parameter ^A	Dietary Treatment						Main effect of level of feeding		
	Control	0-17 M	0-7 <i>ad lib</i>	0-17 <i>ad lib</i>	Minus 7-17 <i>ad lib</i>	<i>P value</i>	Maintenance	<i>Ad lib</i>	<i>P value</i>
Ovulation rate per ewe (all ewes)	1.67 ± 0.082	1.73 ± 0.082	1.74 ± 0.081	1.85 ± 0.083	2.04 ± 0.083	0.113	1.70 ± 0.068	1.87 ± 0.056	0.084
Proportion of ewes with multiple ovulations	0.65	0.67	0.67	0.77	0.83	0.070	0.66	0.76	0.045
Proportion of ewes pregnant	0.67	0.62	0.57	0.68	0.59	0.687	0.65	0.62	0.505
Proportion of all ewes with multiple fetuses	0.23	0.20	0.13	0.14	0.13	0.426	0.21	0.13	0.043
Proportion of pregnant ewes with multiple fetuses	0.34 b	0.32 b	0.22 ab	0.18 a	0.23ab	0.026	0.33	0.21	0.001
No. fetuses/ewe	0.90 ± 0.081	0.83 ± 0.080	0.71 ± 0.080	0.81 ± 0.081	0.73 ± 0.081	0.337	0.87 ± 0.060	0.75 ± 0.049	0.146
No. fetuses/pregnant ewe	1.34 ± 0.062	1.32 ± 0.064	1.23 ± 0.067	1.18 ± 0.062	1.23 ± 0.067	0.347	1.33 ± 0.044	1.21 ± 0.038	0.046
Embryo losses (proportion)									
<i>Total retention</i>	0.36 b	0.38 b	0.24 ab	0.22 ab	0.17 a	0.031	0.37	0.21	0.002
<i>Partial loss</i>	0.29	0.23	0.30	0.43	0.37	0.095	0.26	0.37	0.033
<i>Total loss</i>	0.33	0.37	0.43	0.31	0.42	0.527	0.35	0.39	0.48

a,b: Different letters within rows within analyses indicate means differ significantly ($P < 0.05$).

^A Values are least square means ± the standard error of the least squares mean except for the proportion parameters.

Table 4 Mean lambing performance and lamb survival for ewes fed a control diet or lucerne fed at maintenance or *ad libitum* around the time of artificial insemination (2013 and 2014).

Reproductive parameter ^A	Control	0-17 M	0-7 <i>ad lib</i>	0-17 <i>ad lib</i>	Minus 7-17 <i>ad lib</i>	<i>P value</i>
Lambs born/pregnant ewe	1.32 ± 0.066	1.30 ± 0.067	1.17 ± 0.071	1.22 ± 0.065	1.22 ± 0.071	0.506
Lambs born/ewe inseminated	0.80 ± 0.092	0.79 ± 0.092	0.62 ± 0.091	0.77 ± 0.092	0.66 ± 0.092	0.523
Proportion lamb survival (marked/lamb born)	0.73	0.7	0.78	0.82	0.68	0.689
Lambs marked/ewe inseminated	0.59 ± 0.083	0.55 ± 0.083	0.48 ± 0.082	0.64 ± 0.083	0.47 ± 0.084	0.583
Lambs marked/pregnant ewe	0.92 ± 0.085	0.89 ± 0.087	0.86 ± 0.091	0.98 ± 0.084	0.83 ± 0.092	0.791

^AValues are least square means ± the standard error of the least squares mean except for the proportion lamb survival.

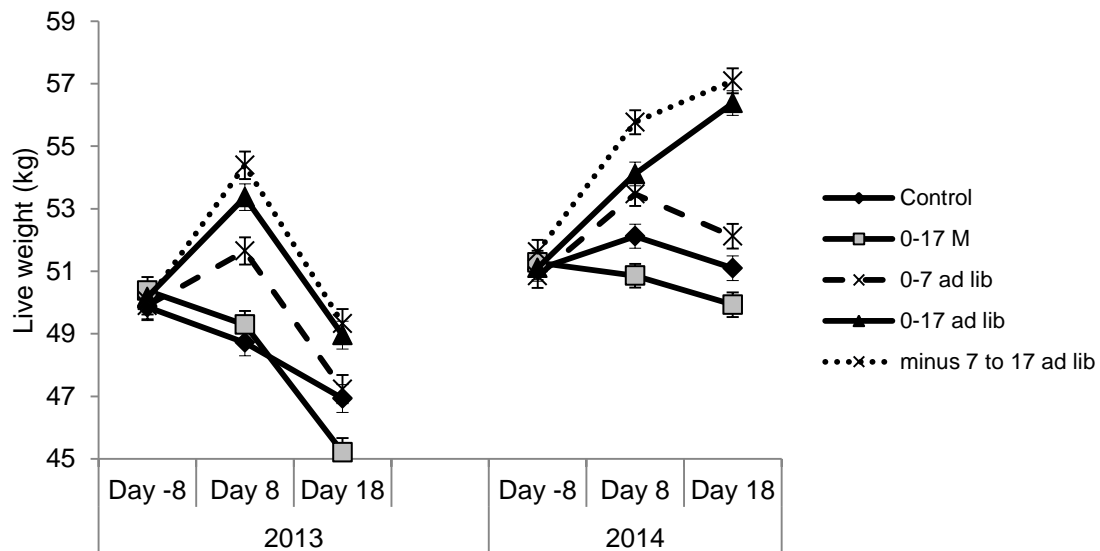


Fig. 1. Mean live weight (kg \pm sem) of ewes in five dietary treatments at Days -8, 8 (unfasted) and 18 (fasted) in 2013 and 2014, using fasted weight at allocation as a covariate.

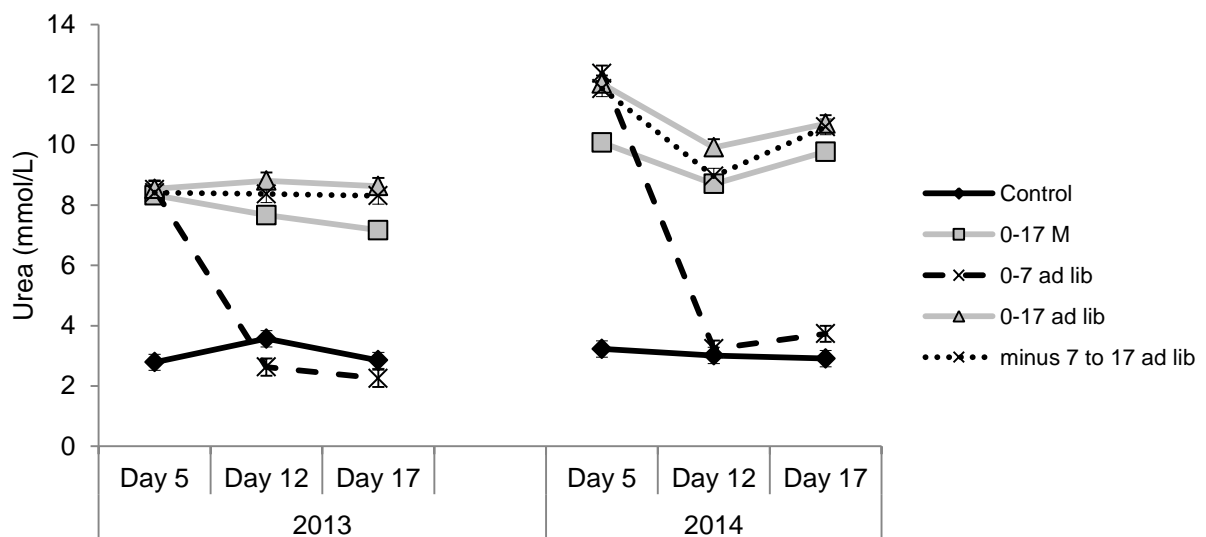


Fig. 2. Mean plasma urea concentration (mmol/L \pm sem) at Days 5, 12 and 17 after artificial insemination in different dietary treatments in 2013 and 2014.

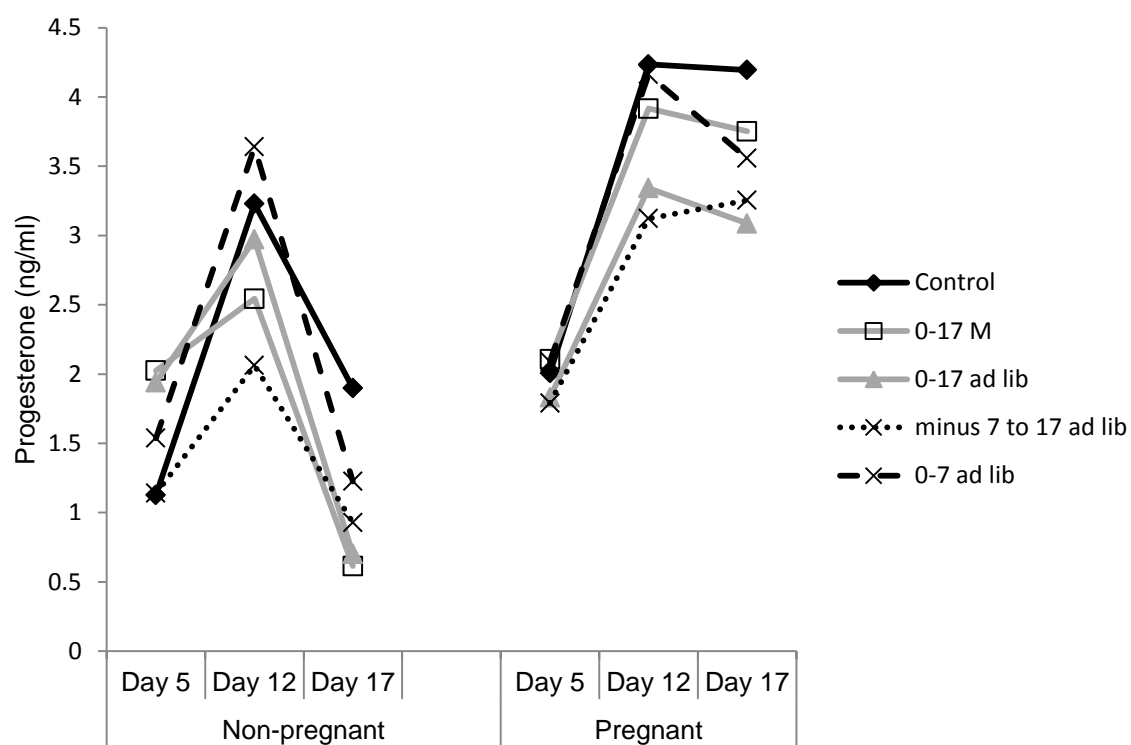


Fig. 3. Mean plasma progesterone concentration (ng/ml) for non-pregnant and pregnant ewes at Days 5, 12 and 17 after artificial insemination in different dietary treatments. Values are backtransformed means (transformed using Log_e transformation prior to analysis).

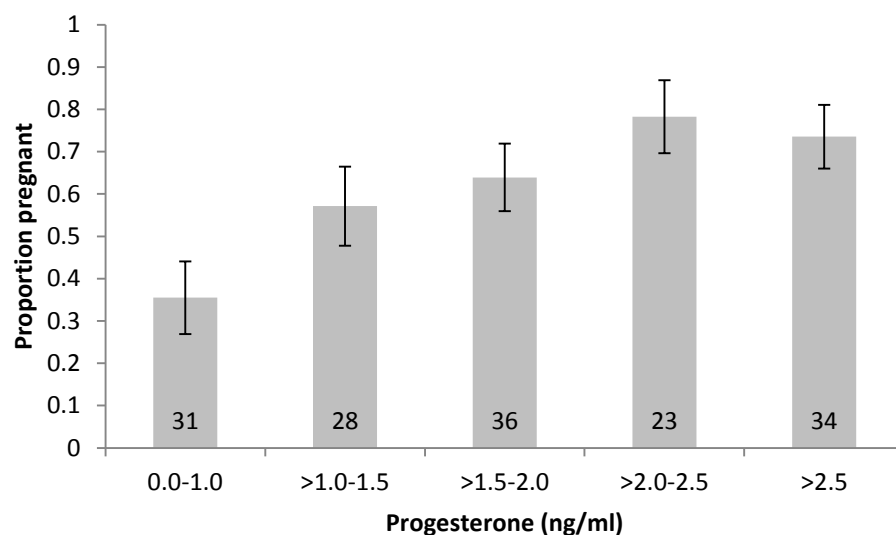


Fig. 4. The proportion of ewes (\pm sem) pregnant for different levels of plasma progesterone at Day 5 after insemination (2013 and 2014). Numbers in columns indicate number of ewes.

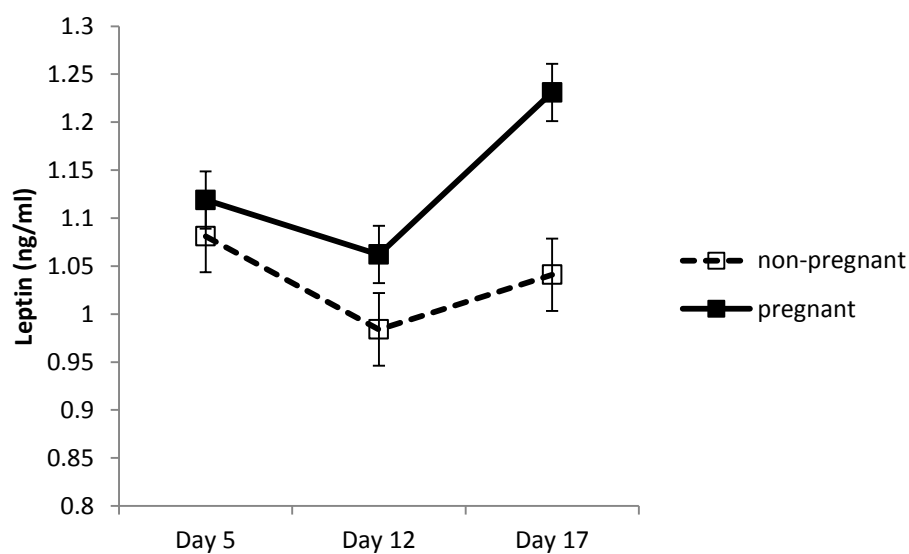


Fig. 5. Mean (\pm sem) plasma leptin concentration (ng/ml) for non-pregnant and pregnant ewes at Days 5, 12 and 17 after insemination (2013 and 2014).

Appendix 7.

Grazing lucerne throughout joining does not reduce reproductive performance in unsynchronised ewes

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Abstract

High levels of nutrition in early pregnancy may increase embryo mortality, negating any benefit of improved nutrition on ovulation rate. Optimum nutritional management for ewes grazing pastures is unclear. This study was conducted to determine whether grazing ewes on live lucerne (*Medicago sativa*) pasture for seven days prior to and throughout joining would result in higher fetal numbers than if ewes were removed at day seven after the commencement of joining, or grazed senescent pasture throughout. Merino ewes (300) were allocated to two replicates of the three treatments, grazing pastures between Days -7 and 36 of an unsynchronised, natural autumn joining. Grazing lucerne to Day 7 of joining resulted in 30% more ($P < 0.05$) fetuses scanned per ewe than grazing dead pasture (1.60 ± 0.07 and 1.31 ± 0.07 , respectively), and 19% more lambs marked per ewe joined. Extending grazing of lucerne past Day 7 of joining did not result in additional fetuses per ewe (1.61 ± 0.06) in comparison with grazing lucerne only to Day 7. Over 80% of ewes mated during the first 14 days of joining, and the proportions of ewes returning to service (0.18 ± 0.022) and of non-pregnant (0.09 ± 0.017) ewes were similar ($P > 0.05$) between all treatments. Grazing naturally cycling autumn-joined ewes on lucerne prior to and during joining is recommended as a means to increase the number of lambs born, although additional gains may not be obtained by grazing past day seven of joining where most ewes mate during the first 14 days.

Keywords sheep, nutrition, flushing, reproduction, embryo mortality, wool

1. Introduction

The development of optimal nutritional strategies in the peri-conceptual period requires consideration of the net effect on lamb production from fertility, ovulation rate, embryo and lamb survival. An increase in nutrition prior to mating, known as flushing, is well known to increase ovulation rates (Scaramuzzi *et al.* 2006) and/or the number of lambs born (Ramirez-Restrepo *et al.* 2005; Viñoles *et al.* 2009). However, pen-feeding studies have also shown that feeding twice maintenance levels of energy (Cumming *et al.* 1975; Parr *et al.* 1987) or high levels of nitrogen or protein (McEvoy *et al.* 1997; Bishonga *et al.* 2006; Meza-Herrera *et al.* 2010) during the peri-conceptual period can result in increased levels of embryo mortality, potentially negating any benefit of higher ovulation rates on the number of lambs born.

However, feeding of above-maintenance levels of energy has not consistently increased embryo mortality. Some studies observed increased embryo mortality (Parr *et al.* 1987; Robertson *et al.* 2014a), while others have not (Wilkins 1997; Athorn *et al.* 2012). The timing of increased nutrition may have an impact, since the sheep embryo is sensitive to the low progesterone levels induced by over-feeding on Days 11 and 12 of pregnancy (Parr 1992), although the rate of increase in progesterone earlier in pregnancy is also important for embryo survival (Ashworth *et al.* 1989). This knowledge has led to strategies where lupin grain supplements have been withdrawn at day 7 after insemination (Viñoles *et al.* 2012) or ewes removed from high-energy pasture (Robertson *et al.* 2014b) seven days after the start of joining in order for flushing to produce increased ovulation rates but minimise the risk of embryo mortality. However, while fetal numbers were reduced in oestrous synchronised and artificially inseminated ewes which were pen-fed lucerne (*Medicago sativa*) *ad libitum* rather than at maintenance to day 17 after insemination (Robertson *et al.* 2014a), fetal numbers were also numerically lower when fed *ad libitum* only to day 7 (Robertson *et al.* unpublished), indicating a possible risk.

The varying impact of high levels of nutrition between studies requires clarification if nutritional strategies are to be effectively applied to increase the number of lambs born in commercial situations. While grazing lucerne before and for the first 7 days of joining is an effective means of increasing the number of fetuses (Robertson *et al.* 2014b), this practice restricts the percentage of ewes in a naturally cycling flock which are flushed in the critical days 10 to 14 of the oestrous cycle (Stewart and Oldham 1986). However, since embryo mortality appears to be increased by pen-feeding lucerne *ad libitum* to day 17 after artificial insemination in oestrous synchronised ewes (Robertson *et al.* 2014a), it is unknown whether access to grazing of large quantities of high quality lucerne beyond day 7 of joining in naturally cycling ewes would increase fetal numbers due to higher ovulation rates in a larger proportion of ewes, or reduce fetal numbers due to an increase in embryo mortality. Therefore, the aim of this study was to evaluate whether fetal numbers could be increased by continuing to graze naturally cycling ewes on live lucerne pasture beyond day 7 of joining in comparison with grazing senescent pasture.

2. Materials and methods

2.1. Location and design

A grazing experiment was conducted with the approval of the Charles Sturt University Animal Ethics committee during 2014 (project 13/088) on a commercial property (34°48'S; 147°26'E) 40 km north of Wagga Wagga, NSW. The experiment evaluated three nutritional treatments implemented from seven days before joining and throughout a five week joining: ewes grazing senesced annual pasture throughout; ewes grazing live lucerne pasture throughout; and ewes grazing live lucerne pasture to day 7 of joining, then senescent pasture for the remainder. Dryland lucerne (*Medicago sativa* cv. Aurora), and senescent pastures based on annual grasses (barley grass (*Hordeum leporinum*), brome grass (*Bromus spp.*)) were used. A randomised design without blocking with two replicates of each treatment was employed.

2.2. Management

A flock of 300 medium framed (50 kg standard reference weight) Merino ewes was used, comprised of two age groups (3.5 and 5.5 years). For one month prior to the experiment, ewes grazed a senescent oat (*Avena sativa*) stubble containing some grain. Where Day 0 (7 March 2014) is the day rams were introduced, on Day -7 the ewes were stratified on age, randomly allocated to replicate and treatment groups (n=50) and placed in treatment paddocks. Non-experimental ewes were also placed in appropriate paddocks to maintain similar numbers of ewes per paddock (n=100) throughout joining. On Day 0, two Merino rams were introduced to each paddock of 100 ewes. Each ram was fitted with a crayon harness (Mating Mark, Rurtec Ltd, Hamilton, New Zealand), and the crayon colour was changed on Day 14 to allow detection of returns to service. The rams were rotated between paddocks within replicates on Days 3, 7, 12, 14, 21, and 28 to prevent ram performance and genetic effects between treatments. The rams were removed from ewes on Day 36, and all ewes grazed as one mob from then until one week prior to lambing. Numbered plates were secured around the neck of ewes to facilitate identification, then for the duration of lambing, the ewes were grazed in replicate groups in two paddocks. After lamb marking, all sheep were grazed as one flock until weaning on 10 November, after which the ewes grazed together until shearing in January. No supplementary feeding of ewes was required during the year.

The lucerne paddock grazed by replicate 2 was changed on Days -4, 3 and 21 to maximise the quantity of lucerne available. The senescent paddocks were sprayed with glyphosate on Days 1 and 16 due to rain causing germination of annual grasses, but continued rain, staggered germination and difficulty in spraying germinating plants covered by large quantities of dead herbage, meant a small quantity of live material was present in both replicates of the senescent pasture throughout much of the joining period. In an attempt to reduce the quantity of live material in replicate 1, the experimental ewes were removed to an alternative senescent pasture on Day 3 and returned on Day 5, during which time the paddock was grazed by a large mob of non-experimental sheep.

2.3. Sheep measurements

The body condition (scale 0 (emaciated) to 5 (obese)) (Jefferies 1961) and live weight of ewes was recorded without fasting at two week intervals on Days -7, 7, 21, 35 and on Day

126 prior to the commencement of lambing. Crayon marks on ewes, indicating mating, were recorded on Days 3, 7, 14, 21, 28 and 35. Trans-abdominal ultrasound was performed 52 days after rams were removed, to determine fetal number and age, by a commercial operator.

Ewes which had been marked with crayon between Days 0 and 3 of joining were identified, and 8 per treatment per replicate randomly selected for blood sampling. Blood samples were collected into lithium heparinised vacutainers from the same 48 ewes on Days 3, 7 and 14 of joining, and stored on ice until plasma was separated by centrifuge. Plasma samples were frozen at -20°C until analysis. On Day 14, fresh blood was immediately analysed for glucose content using an Accu-check Advantage blood glucose meter (Roche Diagnostics Australia Pty Ltd 31 Victoria Avenue, Castle Hill, NSW Australia) and Accu-Chek Performa test strips. Plasma samples which had been frozen were analysed for urea concentration using an enzymatic method via urease and glutamate dehydrogenase (Randox Laboratories Ltd. Crumlin, UK, product # UR 221) (Tiffany *et al.* 1972). Progesterone was measured in duplicate using an Immunotech RIA Progesterone kit IM1188 (Beckman Coulter Gladesville, NSW 2111, Australia). For ewes which were blood sampled, the crayon marks and fetal age were used to determine which ewes became pregnant to Day 3 of joining and did not return to service, so that blood measurements could be associated with pregnancy status.

During lambing, the ewes were checked each morning and afternoon. Lambs were identified with their mothers, tagged, and sex recorded. Dead lambs were removed from paddocks, and lamb survival was recorded at marking on 15 September. Lambs were weighed post-marking on 30 September, and weaned on 9 November.

Midside wool samples were collected post-weaning from 20 ewes per treatment and fleece weights, excluding bellies, for all ewes were recorded at shearing in January. The wool samples were tested in a commercial laboratory (Micron Man Pty Ltd, Bibra Lake, Western Australia) for yield (methods IWTO 33 and 34), fibre diameter (OFDA 100; IWTO 47) and along-staple fibre diameter profiles (OFDA 2000), staple strength (Staple Breaker equipment) and length (IWTO 30).

2.4. Pasture measurements

Pasture measurements were recorded at weekly intervals from Day -8 to the end of joining. Live and dead pasture biomass were visually estimated using the method of Haydock and Shaw (1975). On each sampling occasion, 20 calibration quadrats were cut at ground level with blade shears, and 60 visual estimates per paddock were taken from a zigzag transect through each paddock. The height of live lucerne plants was recorded on each sampling occasion, excluding Day 0. Height to the top of the plant was measured to the nearest cm using a ruler, at 60 locations randomly selected across the same transect used for biomass estimates.

Samples for herbage quality were taken across the same transect using the 'toe-cut' method (Cayley and Bird 1996), with samples cut at ground level using blade shears at 20 points. The samples for each paddock were bulked, then sorted into live and dead. The dead samples were dried in an oven at 60°C to constant weight and retained for nutritive analyses. Live lucerne samples were stripped of leaf and leaf and stem portions dried and weighed to determine the proportion leaf. On the same occasions, pluck samples of live

pasture by species (lucerne, witch grass (*Panicum capillare*), annual grass (mainly barley grass or brome grass) were collected for each paddock when sufficient quantities were available, bulked within paddocks, and dried.

The 'toe-cut' samples of dead pasture, and pluck samples of live lucerne pasture and annual grass, if available, for each paddock on Days -8, 6, 13 and 27 of joining were analysed for nutritive value. Proximate analyses (% DM) were determined using near infra-red reflectance (NIR) spectroscopy with a Bruker multi-purpose analyser (MPA, Bruker Optik GmbH, Ettlingen, Germany) and OPUS software (version 5.1) using calibrations developed by the NSW Department of Primary Industries Feed Quality Service (NSW DPI FQS, Wagga Wagga, New South Wales) as described previously (Packer *et al.* 2011). Crude protein (CP) was estimated from nitrogen (N, CP (% DM) = N (% DM x 6.25)) and metabolisable energy (ME) was estimated from digestible organic matter in the dry matter (DOMD, lucerne ME (MJ/kg DM) = 0.203 DOMD (%) – 3.001; (Anon. 2007)).

2.5. Estimating intake

Pasture intake by the ewes was estimated for the periods Days -8 to 7, and 7 to 36 using GrazFeed™ version 4.1.13 (Freer *et al.* 1997). Measurements of ewe weight and pasture mass, height and nutritive value were used as the basis for input values.

2.6. Statistical analyses

Fetal number per ewe joined excluded data for one ewe which missed scanning. Data from three ewes was not included in lambing performance per ewe joined - two were missing pre-lambing so were not placed in lambing paddocks. A third ewe was sick during joining and removed, so her data was excluded. Data from a further four ewes which died immediately prior to or during the lambing period, due to pregnancy or lambing complications, were included.

Prior to analysis, data were assessed for assumptions of normal distribution and homogeneity using Genstat® 16th (VSN International 2013). Urea and progesterone data were logarithmically transformed prior to analysis using repeated measures, with treatment x Day x pregnancy status as the fixed effects, ewe tag as the subject and Day as the time point. The proportion of ewes raddled, returning to service, with different fetal numbers and lamb survival were analysed using generalised linear mixed modelling using a binomial distribution, with treatment as the model fitted. The logit transformation meant standard errors for backtransformed means were not available. Ewe weight and condition score data were analysed using repeated measures for the joining period, with treatment and Day as the fixed effects, ewe tag as the subject and Day as the time point. Pre-lambing ewe weight and condition, fetal number and lambs born per ewe, day of lambing, lamb weights and wool data were analysed using linear mixed modelling using treatment as the fixed and replicate as the random effects. Plasma glucose concentration was similarly analysed, with one outlying data point removed. The association between fetal number or pregnancy status resulting from mating between Days 0 and 3 of joining and glucose, urea and progesterone concentrations was assessed using linear regression. A P-value of 0.05 was considered significant.

3. Results

3.1. Pastures

The mean quantity of live available herbage in lucerne pastures was at least 400 kg DM/ha except on Day 0 of joining, and increased over the joining period (Fig. 1). For most of the joining period the senescent paddocks also contained a low quantity of live herbage (< 20 kg DM/ha), but a larger quantity was available towards the end of joining. The mean quantity of dead pasture in each paddock remained above 1000 kg DM/ha on all sampling occasions, and was between 1500 and 4000 kg DM/ha except on Day -8 when poor calibration resulted in lower estimates.

Insert Fig 1 here

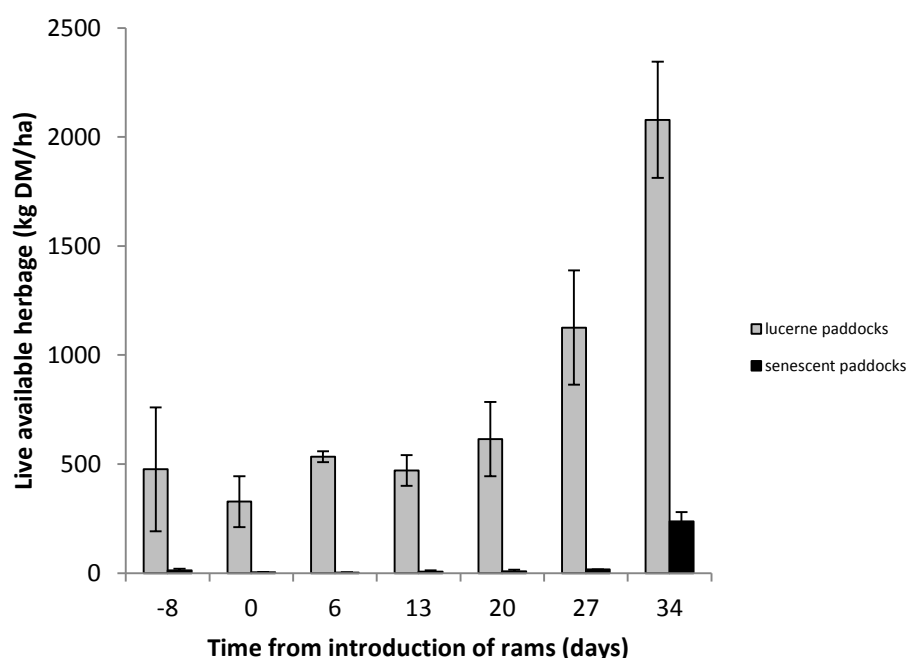


Fig. 1. Mean quantity of live available herbage (kg DM/ha \pm sem) in lucerne and senescent pasture paddocks prior to and following the introduction of rams (Day 0).

The mean height of live lucerne ranged from 22 ± 4.3 cm to 31 ± 5.6 cm across the days sampled. Lucerne initially comprised 98 to 100% of the live herbage in the lucerne paddocks. However, with germination and growth of annual grasses, this declined to between 70 and 98% between Days 6 and 20 of joining, and between Days 27 to 34 ranged between 48 and 74%. Annual grasses were the only live herbage in senescent pasture paddocks.

The proportion leaf of live lucerne remained high throughout joining in all lucerne paddocks, and ranged between 40 and 65%. The metabolisable energy, protein and water soluble carbohydrate content of plucked lucerne samples was relatively high, and that of dead grass

low, throughout joining (Table 1). Because samples of live annual grass were not available on all sampling days in each paddock, means are presented for all days combined.

Table 1 Metabolisable energy (MJ ME/kg DM), crude protein (CP %), neutral detergent fibre (NDF %) and water soluble carbohydrate (WSC %) of lucerne and dead grass pasture between Day -8 and 27 from introduction of rams (Day 0) (mean \pm sem).

	Paddock	Plant type	Day-8	Day 6	Day 13	Day 27
ME (MJ/kg DM)	Lucerne	Live lucerne	11.3 \pm 0.65	14.6 \pm 0.20	12.6 \pm 0.10	14.1 \pm 0.05
	Senescent	Dead grass	5.8 \pm 0.25	5.4 \pm 0.15	5.3 \pm 0.15	5.4 \pm 0.25
	Lucerne + Senescent	Live grass	12.7 \pm 0.50 (combined days)			
CP (% of DM)	Lucerne	Live lucerne	19.9 \pm 1.15	32.6 \pm 0.65	32.9 \pm 2.60	31.8 \pm 1.70
	Senescent	Dead grass	2.2 \pm 0.20	<2.0	<2.0	2.7 \pm 0.65
	Lucerne + Senescent	Live grass	27.4 \pm 2.53 (combined days)			
NDF (% of DM)	Lucerne	Live lucerne	37.0 \pm 2.00	27.5 \pm 0.50	30.5 \pm 0.50	30.5 \pm 1.50
	Senescent	Dead grass	77 \pm 0.50	81 \pm 1.00	80 \pm 2.00	79 \pm 2.00
	Lucerne + Senescent	Live grass	52.6 \pm 2.25 (combined days)			
WSC (% of DM)	Lucerne	Live lucerne	3.4 \pm 0.45	3.4 \pm 0.15	2.5 \pm 0.15	7.6 \pm 1.53
	Senescent	Dead grass	1.2 \pm 0.19	2.3 \pm 1.29	2.1 \pm 1.29	2.0 \pm 0.98
	Lucerne + Senescent	Live grass	4.9 \pm 0.81 (combined days)			

3.2. Ewe weight and condition

The mean weight of ewes in all treatments (54.1 \pm 0.30) was similar ($P>0.05$) at allocation (Day -7) (Fig. 2a). Weight initially declined for ewes in the senescent pasture treatment, but they maintained ($P>0.05$) weight between Day 7 and 36. Weight was maintained when ewes grazed lucerne from Day -7 to 7, however, thereafter ewes grazing lucerne gained weight such that they were 10 kg heavier ($P<0.05$) than ewes in the senescent pasture treatment at Day 36. The maximum weight gain recorded was 300 g/day between Days 7

and 21. Weight declined ($P < 0.05$) between Days 7 and 36 for those ewes which were removed from lucerne to senescent pasture at Day 7

Insert Fig. 2 here

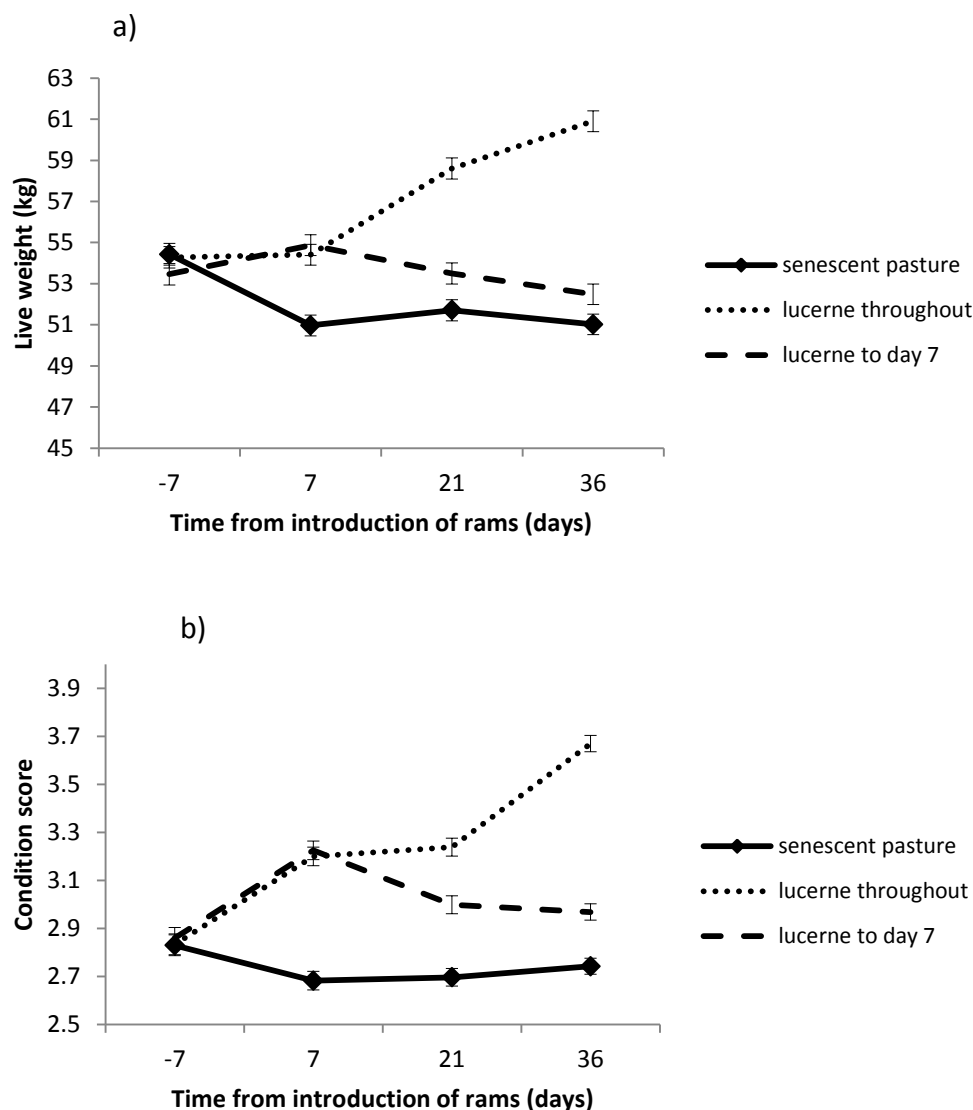


Fig. 2. Mean a) unfasted live weight (kg \pm sem) and b) condition score (score \pm sem) of ewes in three treatments prior to and following the introduction of rams (Day 0).

The mean condition score of the ewes at allocation (Day -7) (2.8 ± 0.03) was similar ($P > 0.05$) between treatments (Fig. 2b). The condition score of ewes increased ($P < 0.05$) to Day 7 of joining and thereafter if ewes grazed lucerne. By the end of joining (Day 36), ewe condition score had been maintained ($P < 0.05$) in the senescent pasture treatment (2.7 ± 0.03), increased ($P < 0.05$) for ewes grazing lucerne throughout (3.7 ± 0.04), with a smaller increase ($P < 0.05$) in condition for ewes removed from lucerne at Day 7 (3.0 ± 0.03).

Pre-lambing, ewes which had grazed lucerne throughout joining were heavier and in fatter condition ($P < 0.05$) than ewes which had grazed senescent pasture or lucerne to Day 7 of joining (67.5 ± 0.80 kg and 3.3 ± 0.06 , 61.3 ± 0.80 kg and 3.0 ± 0.06 , and 62.8 ± 0.79 kg and 3.1 ± 0.06 , respectively).

3.3. Estimated pasture intake

The daily weight changes estimated by GrazFeed for the pasture conditions available differed from those calculated from the observed unfasted weights (Table 2). The estimated energy intake was submaintenance between Days -7 and 7 for ewes grazing senescent pasture. The estimated energy intake of ewes grazing lucerne pasture was well above maintenance requirements before Day 7, with 1.9 times maintenance requirements consumed between Day 7 and the end of joining. The estimated energy intake required to achieve the observed weight changes is also shown in Table 2, with zero intake for ewes grazing senescent pasture between Days -7 and 7.

Insert Table 2 here

Table 2 Estimated daily weight change (g) and intake of dry matter (DM), metabolisable energy (MJ ME) and protein predicted by GrazFeed for ewes grazing dead pasture or lucerne between Days -7 and 7, and Days 7 and 36 relative to the introduction of rams.

	Senescent pasture		Lucerne throughout		Lucerne Day 7	
	Day -7 to 7	Day 7 to 36	Day -7 to 7	Day 7 to 36	Day -7 to 7	Day 7 to 36
Weight change (g/day)						
GrazFeed estimate	-72	78	117	163	117	72
Observed estimate	-247	0	9	224	101	-82
Intake limit (kg DM/day)	1.36	1.40	1.41	1.50	1.41	1.38
Intake (kg DM/day)	0.84	1.24	1.39	1.49	1.39	1.22
ME intake (MJ/day)	6.7	13.5	15.9	17.3	15.9	13.2
ME required for maintenance (MJ/day)	8.8	8.0	8.8	9.2	8.8	8.2
Protein intake (g/day)	97	318	312	490	312	292
Protein intake surplus to use (g/day)	35	183	151	314	151	161
ME intake to achieve observed weight change (MJ/day) (fed)	0	10.8	7.4	13.5	13.0	4.2
ME required for maintenance (MJ/day) (fed)	6.7	6.7	6.8	6.4	7.3	6.4
ME intake relative to maintenance (fed) (%)	-	161	109	211	178	66

3.4. Reproduction

As indicated by crayon marks, more than 80% of ewes were mated during the first 14 days of joining, and up to 100% over the 36 days of joining, with no differences between treatments (Table 3). The proportion of ewes which returned to service was not higher ($P>0.05$) when ewes grazed lucerne throughout joining or to Day 7 of joining in comparison with ewes grazing senescent pasture. The proportion of ewes which were not pregnant at scanning was similar ($P>0.05$) between treatments, but the proportion of ewes bearing twin fetuses was increased ($P<0.05$) by grazing lucerne compared with senescent pasture. Further increases in the proportion of ewes with multiples, or the mean number of fetuses

per ewe scanned, were not ($P>0.05$) obtained by grazing lucerne throughout joining, compared with only grazing to Day 7. Only four ewes were scanned with triplet fetuses, and these all occurred in the lucerne to Day 7 treatment.

For ewes which were raddled after Day 14 of joining (including those not raddled previously and those returning to service), the proportion of ewes with multiple fetuses, for all and for pregnant ewes, was similar ($P>0.05$) between treatments. For ewes which returned to service, fetal numbers were 0.3 lower ($P<0.05$) per ewe than for ewes which did not return, but there was no reduction if non-pregnant ewes were excluded (1.66 ± 0.070 and 1.58 ± 0.100 for those not returning and returning, respectively), with no interaction ($P>0.05$) with treatment. However, the number of ewes returning to service which were scanned as pregnant was only 14 to 16 per treatment, making it difficult to detect treatment differences in this group.

Insert Table 3 here

Table 3 Mean (\pm sem) reproductive performance of ewes in three treatments.

	Senescent pasture	Lucerne throughout	Lucerne to Day 7	P-value
Proportion raddled to Day 14	0.87	0.92	0.83	0.172
Proportion returning to service	0.19	0.15	0.19	0.684
Proportion non-pregnant	0.12	0.06	0.09	0.341
Proportion with multiple fetuses				
Of ewes joined	0.43 a	0.67 b	0.65 b	0.001
Of pregnant ewes	0.49 a	0.71 b	0.72 b	<0.001
No. fetuses				
Per ewe joined	1.31 a \pm 0.095	1.61 b \pm 0.095	1.60 b \pm 0.095	0.002
Per pregnant ewe	1.49 a \pm 0.081	1.71 b \pm 0.080	1.76 b \pm 0.081	<0.001

a,b: Different letters within rows indicates means differ significantly ($P<0.05$).

3.5. Lamb production

The mean date of lambing was similar ($P=0.182$) between treatments, occurring 13.3 ± 0.91 , 13.3 ± 0.87 and 15.3 ± 0.88 days from the start of lambing for treatments senescent pasture, lucerne throughout, and lucerne to Day 7, respectively. While the number of lambs born per ewe joined was at least 30% higher ($P<0.001$) for ewes in treatments grazing lucerne compared with senescent pasture (Table 4), the number of lambs marked per ewe joined

was 18 or 19% higher ($P=0.04$) for the lucerne treatments in comparison with the senescent pasture treatment.

The proportion of lambs surviving to marking was similar ($P>0.05$) between treatments, both without (Table 4) and with birth type included in the model. The proportion survival of triplets (0.48) was lower ($P=0.003$) than that of twins (0.75) and singles (0.83), although there were no triplets born in the senescent pasture treatment. Triplets were born in both lucerne treatments, some of which had not been detected at pregnancy scanning. For lambs which died, the weight of twins at death was similar between treatments (Table 4). There were insufficient dead singles for comparison. The mean weight of lambs at marking was similar between treatments when birth type was excluded from the model. The marking weight of lambs born as singles (17.2 ± 0.39 kg) was higher ($P<0.001$) than that of twins (13.7 ± 0.22 kg) or triplets (14.64 ± 1.05 kg).

Insert Table 4 here

Table 4 Mean (\pm sem) production of lambs born to ewes in three treatments.

	Senescent pasture	Lucerne throughout	Lucerne to Day 7	P-value
Lambs born per ewe joined ^A	1.30 a \pm 0.070	1.67 b \pm 0.069	1.63 b \pm 0.70	<0.001
Proportion lamb survival ^A	0.78	0.74	0.74	0.685
No. lambs marked per ewe joined	0.96 a \pm 0.060	1.14 b \pm 0.060	1.15 b \pm 0.060	0.040
Marking weight (kg)	15.3 \pm 0.43	15.0 \pm 0.40	14.6 \pm 0.41	0.509
Dead weight of twins (kg)	5.1 \pm 0.55	4.7 \pm 0.52	4.7 \pm 0.53	0.977

a,b: Different letters within rows indicates means differ significantly ($P<0.05$).

^ALambs born is calculated from scanned fetal number where actual lambs born was not observed (ewe didn't lamb or abandoned lambs before tagging); Lamb survival excludes 12 lambs which could not be identified to ewes and therefore treatment.

3.6. Blood parameters

Glucose, urea and progesterone concentrations were similar ($P>0.05$) between ewes remaining pregnant from the first three days of joining and those that were not. There were no significant interactions between pregnancy status and treatment or day of sampling for these measures. Linear regression showed that fetal number or pregnancy status resulting from mating between Days 0 to 3 of joining were not ($P>0.05$) associated with glucose, progesterone or urea concentrations or the change in progesterone levels between Days 3 and 14 of joining.

The mean glucose concentration of plasma at Day 14 was higher ($P<0.001$) for ewes grazing lucerne (4.9 ± 0.16 mmol/L) compared with ewes which had been removed from

lucerne on Day 7 or those grazing senescent pasture throughout (4.0 ± 0.10 mmol/L), which were similar ($P>0.05$).

The mean plasma urea concentration for ewes grazing lucerne throughout joining was more than twice ($P<0.001$) that of ewes grazing senescent pasture on each day sampled (Fig. 3), and the level of urea in both treatments remained constant ($P>0.05$) over time. Urea concentrations were similar ($P>0.05$) for ewes which grazed lucerne only to Day 7 of joining to that of other ewes grazing the same type of pasture on the same day.

Progesterone levels were lowest ($P<0.05$) on Day 3 of joining, increasing to Day 7 and were highest ($P<0.05$) at Day 14 of joining (Fig. 3). Progesterone concentrations were higher ($P=0.002$) for ewes grazing senescent pasture (3.14 ± 1.10 ng/ml) than for ewes grazing lucerne either to Day 7 (2.08 ± 1.10 ng/ml) or throughout joining (2.00 ± 1.10 ng/ml). Plasma progesterone concentrations did not show an interaction ($P>0.05$) between day of sampling and treatment. The increase in progesterone between days 3 and 14 of joining was similar ($P=0.09$) for the three treatments: senescent pasture 5.75 ± 0.687 ng/ml; lucerne throughout 3.59 ± 0.666 ng/ml and lucerne to Day 7 4.42 ± 0.687 ng/ml. The fold change between Days 3 and 7 was also similar ($P>0.05$) between treatments: 4.4 ± 0.54 ; 4.0 ± 0.52 , 4.0 ± 0.54 for senescent pasture, lucerne throughout, and lucerne to Day 7, respectively.

Insert Fig 3. here

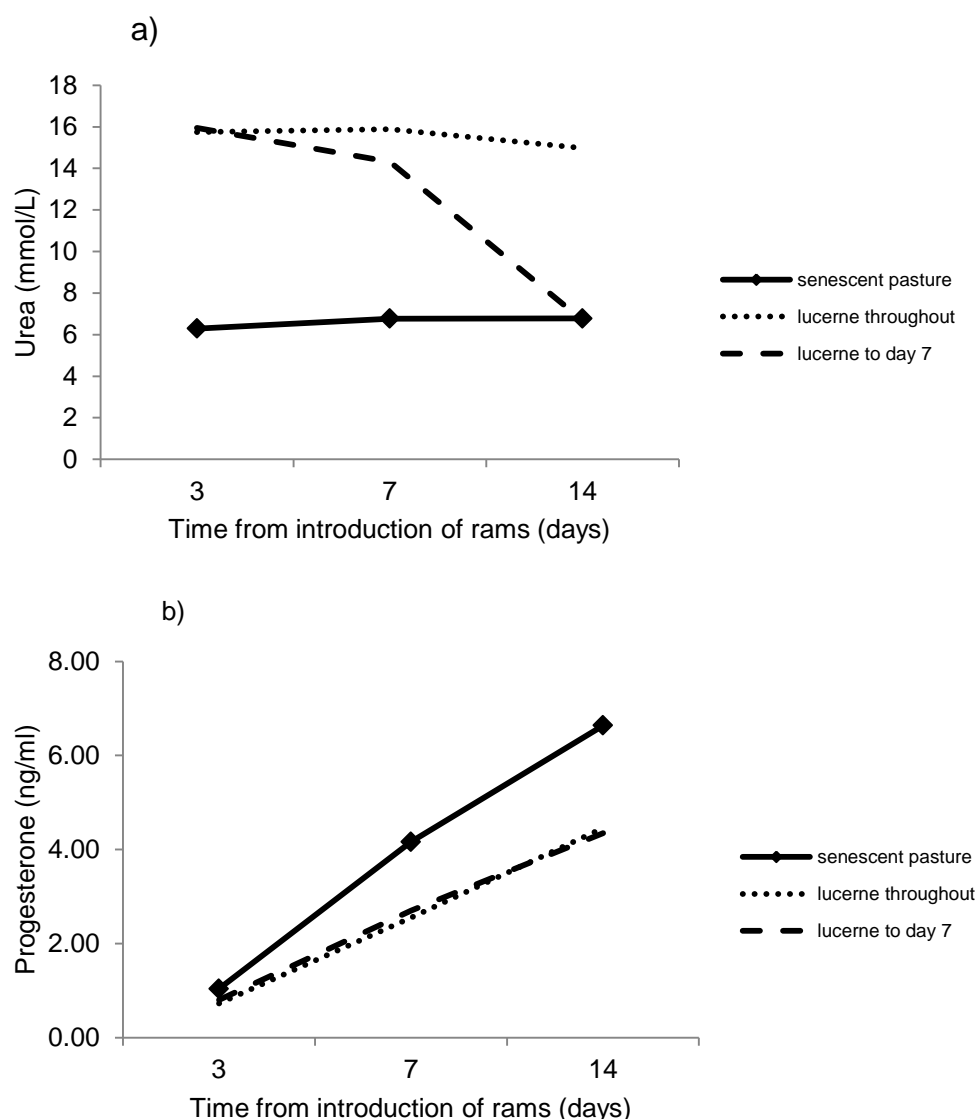


Fig. 3. Mean (\pm sem) plasma a) urea concentration (mmol/L) and b) progesterone concentration (ng/ml) for ewes in three treatments on Days 3, 7 and 14 after introduction of rams. Means are backtransformed.

3.7. Wool production

The mean greasy fleece weight, yield, fibre diameter, staple length, and staple strength of wool was similar ($P > 0.05$) between treatments, with overall means shown in Table 5. However, fleece weights tended ($P = 0.057$) to be higher for ewes grazing lucerne throughout joining (5.1 ± 0.11 kg) compared with those grazing dead pasture (4.7 ± 0.11 kg) or lucerne to Day 7 of joining (4.8 ± 0.11 kg). Staple strength tended ($P = 0.07$) to be higher for ewes in the dead pasture treatment (34.0 ± 1.13 N/ktex) compared with the other treatments (≤ 31.3 N/ktex). The along-staple co-efficient of variation in fibre diameter was lower ($P = 0.002$) for

ewes in the dead pasture treatment ($4.9 \pm 0.38\%$) than if they had grazed lucerne throughout joining ($6.6 \pm 0.38\%$) or to Day 7 of joining ($6.1 \pm 0.38\%$).

Insert Table 5 here

Table 5 Mean (\pm sem) wool characteristics of ewes.

	Mean
Greasy fleece weight (kg)	4.8 ± 0.06
Yield (%)	75 ± 0.4
Fibre diameter (microns)	19.6 ± 0.48
Staple length (mm)	72 ± 0.8
Staple strength (N/ktex)	32 ± 0.7
Minimum along-staple fibre diameter (microns)	17.9 ± 0.35
Maximum along-staple fibre diameter (microns)	21.7 ± 0.51

4. Discussion

Fetal numbers were increased by 30% and lambs marked per ewe joined by 19% as a result of grazing ewes on live lucerne between Day -7 and Day 7 of joining rather than senescent pasture, but there was no further benefit from grazing lucerne beyond Day 7 of joining. This is likely due to approximately 90% of ewes being mated during the first 14 days, with those mated up to day 10, as a minimum, of joining being flushed in both lucerne treatments. An increase in embryo mortality for ewes which grazed lucerne past Day 7 was not evident from the proportion of non-pregnant ewes, or from returns to service. Furthermore, when non-pregnant ewes were excluded, both fetal number per ewe and the proportion of ewes with multiples did not decline in ewes which returned to service, compared with those which did not, providing some evidence that partial failure of multiple ovulations was unlikely to have been increased by grazing lucerne past Day 7 of joining.

The proportion of pregnant ewes with multiple fetuses was not reduced by lucerne in this grazing study, which contrasts with a previous pen-feeding study where fresh lucerne pasture fed *ad libitum* to oestrous synchronised and artificially inseminated ewes for 17 days after insemination reduced the proportion with multiples from 34 to 18% (Robertson *et al.* 2014a) (Robertson *et al.*, unpublished). In the current study it is unlikely intake was not high enough to cause embryo mortality since estimated intake was at near maximum levels, and the estimated energy intake was 1.9 times maintenance requirements, similar to levels known to cause embryo mortality (Parr 1992), and greater than those associated with

reduced multiple fetuses (Robertson *et al.* 2014a) (Robertson *et al.*, unpublished). The high energy intakes are consistent with the actual live weight gain of ewes, which were variable between weeks probably due to measuring non-fasted weights. While live weight gains prior to Day 7 of joining were below 110 g/day, ewe condition did increase, and for the treatment grazing lucerne throughout joining, ewe weight gains were large (300g/day) for all ewes during the period (days 11 and 12 of pregnancy) when excess nutrition is reported to be most detrimental (Parr 1992). Any potential adverse effect of lucerne is unlikely to have been prevented by the live annual grass component of lucerne pastures, since the response appears to be due to the energy intake, more than the type of feed (Robertson *et al.* 2014a) (Robertson *et al.*, unpublished).

A reduction in fetal numbers as a result of possible weight loss in ewes grazing senescent pasture restricting ovulation rate, rather than ovulation rate being increased by lucerne, is considered unlikely. Ovulation rate was not reduced in ewes fed sub-maintenance diets in the study by Cumming (1977) and the ovulatory response to increased nutrition appears to be independent of live weight at mating (Killeen 1967). Large ovulatory responses can be obtained from ewes grazing relatively low quantities of live pasture (350 kg DM/ha) (King *et al.* 2010), and the effectiveness of short-term flushing with lucerne to Day 7 of joining in the current study is consistent with previous grazing studies (Robertson *et al.* 2014b). In both of those studies, the ewes on the senescent pasture had a slight weight gain (about 1 kg) compared with 2-3 kg gains on lucerne. The larger increase in fetal numbers in the current study (30 per 100 ewes) compared with the previous grazing study (18 per 100 ewes) (Robertson *et al.* 2014b) may be partially explained by the three week later joining, with ovulation rate increasing towards the peak of the breeding season (Dun *et al.* 1960). The apparent much larger increase in ovulation rate in the current study than the 10% recorded in the study of King *et al.* (2010) is most likely due to the low leaf content and quality of drought-affected lucerne, and insufficient quantity of lucerne being available for the duration of the flushing period in that study.

The apparent lack of a detrimental effect of elevated nutrition in early pregnancy on reproductive outcomes in the current study, in contrast to other studies, may be associated with the use of naturally cycling, rather than synchronised and artificially inseminated ewes. The use of gonadotrophins is known to reduce the number of transferable embryos from superovulated cattle (Greve *et al.* 1995) and also causes abnormal oocyte development in sheep (Moor *et al.* 1985). However, a 12% increase in embryo mortality has been reported in unsynchronised, naturally mated ewes as a result of feeding 500 g/day lupins to ewes already rapidly gaining weight while grazing pasture (Brien *et al.* 1977). The effect was associated with a reduction in plasma progesterone concentrations (Brien *et al.* 1981).

Circulating plasma progesterone concentrations in ewes were reduced by grazing lucerne. However, progesterone levels were not reduced to the minimum concentration of 2 ng/ml on Day 12 after mating suggested by Parr *et al.* (1987) as the threshold above which embryo mortality is minimised, and this is proposed as a reason why access to high quality lucerne in early pregnancy did not appear to increase embryo mortality in the present study. The importance of adequate progesterone levels at different stages of pregnancy has been described for both sheep (Ashworth *et al.* 1989) and cattle (Diskin *et al.* 2012) (Kenyon *et al.* 2013). However, the variation in progesterone levels between different flocks and the management factors which producers could employ to maintain appropriate levels are not clear.

The plasma urea concentrations measured in ewes grazing lucerne were more than twice the level (6 mmol/L) known to be associated with increased mortality (Bishonga *et al.* 2006), yet there was apparently no adverse effect. The lack of association between plasma urea and reduced performance is perhaps not surprising since high protein/urea levels are more likely to be detrimental when the animal is in negative energy balance, or if the protein level fed is extreme (Velazquez 2011). The ewes in the current study were gaining weight so in positive energy balance while grazing lucerne. In addition, protein supplied from lucerne takes longer to degrade and be absorbed into the blood, such that the peak in plasma ammonia or urea is likely to be less than that arising from use of urea supplements (Bishonga *et al.* 2006), as has often been used in embryo studies. The lack of adverse effect is consistent with other grazing studies which have shown an increase in lamb production (Ramirez-Restrepo *et al.* 2005) or no increase in embryo mortality (Kenny *et al.* 2001) from grazing pastures with higher protein levels. While a reduction in pregnancy rate and an increase in embryo mortality due to grazing lucerne compared with grass pastures was reported by Smith *et al.* (1979), in that study, the rams were not rotated between treatments, and as production was not reduced on all lucerne treatments, it is possible that ram effects were the cause.

Blood glucose levels are positively associated with the level of feed intake (Parr and Williams 1982), but in the current study there were not large differences in glucose levels despite large differences in the quantity of live pasture. High glucose levels in *in vitro* culture of embryos are known to reduce survival of mouse embryos (Bermejo-Alvarez *et al.* 2012), and hyperglycemia either as a result of high energy intake or low protein intake (Kwong *et al.* 2000; Fernandez-Twinn *et al.* 2003) (Meza-Herrera *et al.* 2010) may adversely affect reproduction. However, higher glucose levels did not appear to have an adverse impact in the current study.

The sex ratio of lambs has previously been found to be altered by pen-feeding lucerne pasture before artificial insemination (Robertson *et al.* 2014a). The effect of lucerne on lamb sex from that study and the current grazing study will be reported in detail in a separate paper, with possible causative mechanisms discussed (Clayton *et al.*, unpublished).

Economically important wool traits were not adversely affected by the grazing treatments and resultant differing fetal numbers. This is consistent with a limited effect of reproduction on wool variables under suitable nutritional conditions (Robertson *et al.* 2000), although under poorer pasture conditions, the value of wool produced may be reduced as a result of the larger nutritional demands of ewes bearing twin fetuses. Although the staple strength of wool was not reduced by the lucerne treatments, the greater variability of along-staple fibre diameter for ewes which grazed lucerne indicates that there may be an increased risk of lower staple strength if the time of shearing differed (Arnold *et al.* 1984) due to alteration of the fibre diameter profile. However, under the conditions of this study, the combined value of wool and lambs produced could be expected to be much higher for the lucerne compared with the senescent pasture treatment.

Conclusion

It is concluded that grazing naturally cycling ewes on lucerne pasture is an effective means of increasing lamb marking percentages, with no indication in this study of an increased risk of embryo mortality. Producers can most efficiently use limited pasture by only grazing lucerne from 7 days before to day 7 of joining, for joining during the breeding season where most ewes mate in the first 14 days. An increase in fetal numbers may be possible from

grazing lucerne past day 7 of joining if less ewes were mated early in the joining period, or a larger proportion returned to service. Attention to management for lamb survival is important to maximise the benefits of increased fetal numbers. The factors which contribute to lucerne increasing reproductive failure in artificially inseminated/synchronised situations require further clarification.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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