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Title: Reproductive performance of ewes grazing lucerne during different periods around mating

Journal: Animal Reproduction Science

ISSN: 1873-2232 **Year:** 2015 **Pages:** 62 - 72

Volume: 162 **Issue:**

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 i ...

URLs:

FT: http://dx.doi.org/10.1016/j.anireprosci.2015.05.001

PL: http://primo.unilinc.edu.au/primo_library/libweb/action/dlDisplay.do?vid=CSU2&docId=dtl_csu80074

No reduction in the reproductive performance of unsynchronised ewes grazing lucerne throughout joining

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Abstract

High levels of live lucerne pasture or other high quality feed in early pregnancy may increase embryo mortality, negating any benefit of improved nutrition on ovulation rate. 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 if ewes 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 senescent 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, suggesting no differences between treatments in embryo mortality. 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 (Bishonga et al., 2006; McEvoy et al., 1997; 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.

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., 2015), while others have not (Athorn et al., 2012; Wilkins, 1997). 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). Knowledge that the timing of high energy intake alters the level of embryo mortality 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., 2014) seven days after the start of joining in order for flushing to produce increased ovulation rates but minimise the risk of embryo mortality.

The varying impact of high levels of nutrition between studies requires clarification if nutritional strategies, such as flushing on high energy/protein pastures, 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., 2014), 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). Furthermore, 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., 2015), it is unknown whether access to grazing of ample amounts 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 the overall number of fetuses due to an increase in embryo mortality. Therefore, the aim of the current study was to evaluate whether fetal numbers were increased by continuing to graze naturally cycling ewes on ample amounts of high quality live lucerne pasture beyond day 7 of joining in comparison with grazing senescent pasture. Lamb survival and marking weights, and the wool production of ewes was also evaluated to all comparison of overall effects of the treatments on flock productivity.

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 (34’48ºS; 147’26ºE) 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; sown in 2006 or 2011), 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 (54.1 ± 0.30 kg at allocation) 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 in each of six groups) and placed in treatment paddocks. Four paddocks were used at any one time, two lucerne (15 to 32 ha) and two senescent (10 to 20 ha) pasture paddocks. Ewes grazing lucerne to either day 7 or throughout joining grazed the same lucerne paddock within replicates, and those removed from lucerne were placed in the same paddock as those only grazing senescent pasture, within replicates. 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 differences 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 (tail docking, castration, ear-marking, vaccination) on 15 September, all sheep were grazed as one flock until weaning on 9 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 ensure the live lucerne available contained a high proportion of leaf (> 40%). 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 order 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 prior to the commencement of lambing (Day 126). 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^{o}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 by or on 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. 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 senesced. The senesced samples were dried in an oven at 60^{o}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, dried and retained for nutritive analyses.

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. Intake estimates

Mean daily 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, and one ewe allocated to the senescent pasture treatment was removed from the paddock during joining due to illness. Data from a further four ewes which died immediately prior to or during the lambing period, due to pregnancy or lambing complications, were included in analyses of lambs born and marked per ewe joined (lambs born being 0 if the ewe died before obvious signs of being in the birthing process), but were excluded from analyses of gestation length if they had died before parturition. For the three ewes which died prior to parturition being evident, their unborn lambs were excluded from analyses of lamb survival, For the fourth dead ewe, the lamb was born before the ewe died, so was included in analyses of lamb survival.

Prior to analysis, data were assessed for assumptions of normal distribution and homogeneity using Genstat® 16th (VSN International, 2013). Analyses with repeated measurements used co-variance to allow for repeated measurements on the same ewe, using the model with the minimum deviance. 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 and replicate as the random effect. 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. Ewe weight and condition score prior to lambing, 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 effect. The concentration of glucose in plasma was analysed similarly, 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 least on Day 0 of joining (327 kg DM/ha) 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 senescent pasture in each paddock remained above 1500 kg DM/ha except on Day -8 when poor calibration resulted in lower estimates (data not shown).

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 senesced 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 across all paddocks.

3.2. Ewe weight and condition

The mean unfasted weight of ewes in all treatments (54.1 ± 0.30 kg) 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 in the lucerne throughout treatment. Weight declined ($P<0.05$) between Days 7 and 36 for those ewes which were removed from lucerne to senescent pasture at Day 7, but they remained at least 1.5 kg heavier than ewes in the senescent pasture treatment between Day 7 and the end of joining.

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. Between Days -7 and the end of joining (Day 36), ewe condition score was 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 better 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 estimated energy intake was sub-maintenance between Days -7 and 7 for ewes grazing senescent pasture (Table 2). The estimated energy intake of ewes grazing lucerne pasture was 1.8 times maintenance requirements before Day 7, with 1.9 times maintenance requirements consumed between Day 7 and the end of joining.

3.4. Reproduction

As indicated by crayon marks, more than 80% of ewes were mated during the first 14 days of joining (Table 3), and up to 100% over the 36 days of joining, with no differences ($P>0.05$) between treatments. 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 multiple 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 were all in the lucerne to Day 7 treatment.

For ewes which were marked with crayon 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 ($P=0.004$) 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 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.

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 ewes grazing 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 (single or multiple) included in the model, and there was no interaction between treatment and birth type. Triplets were born in both lucerne treatments, some of which had not been detected at pregnancy scanning. No triplets were born in the senescent pasture treatment, so birth class was evaluated in a separate analysis; the proportion survival of triplets (0.48) was lower (P=0.006) than that of twins (0.75) and singles (0.83). The mean weight of lambs at marking was similar between treatments when birth type was either included or 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).

3.6. Blood parameters

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 throughout (4.9 ± 0.13 mmol/L) compared with ewes which had been removed from lucerne on Day 7 (4.0 ± 0.12 mmol/L) or those grazing senescent pasture throughout (4.0 ± 0.12 mmol/L). Glucose concentrations were similar for ewes found to be pregnant or not pregnant at scanning, and there was no interaction between treatment and pregnancy status.

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. 3a), 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. Urea concentrations were similar between pregnant and non-pregnant ewes, and there was no interaction between treatment and pregnancy status.

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. 3b). 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 (percentage increase/100) 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.

3.7. Wool production

The mean greasy fleece weight, yield, fibre diameter, staple length, and staple strength of wool was not different (P>0.05) between treatments, with means shown in Table 5. The along-staple co-efficient of variation in fibre diameter was lower (P=0.002) for ewes in the senescent pasture treatment than if they had grazed lucerne throughout joining or to Day 7 of joining.

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. An increase in embryo mortality for ewes which grazed lucerne past Day 7 was not evident based on the proportion of non-pregnant ewes, or from returns to service. That fetal numbers were not increased by continuing to graze lucerne after day 7 of joining 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. The proportion of ewes mating or re-mating after this time was small and likely insufficient to markedly alter fetal numbers. Producers could maximise the number of mobs of ewes flushed, or more efficiently use a limited feed resource, by restricting grazing of lucerne to 7 days before and the first 7 days of joining during the natural breeding season. .

The proportion of pregnant ewes with multiple fetuses was not reduced by access to ample, high-quality lucerne in the current 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., 2015). In that study, the reduction in multiple fetuses was attributed to the high intake levels. In the current study it is probable that intake was high enough to cause embryo mortality since estimated intake from the lucerne pasture was 1.9 times maintenance requirements (Table 2), similar to levels known to cause embryo mortality (Parr, 1992), and greater than levels associated with a reduction in multiple fetuses (Robertson et al., 2015). The estimated high energy intakes (via GrazFeed) are consistent with the actual live weight gain and condition score change of ewes. They are also consistent with calculations of energy intake from the weight gain observed using published feeding standards (SCA, 1990). These calculations indicate an energy intake of over twice maintenance between Days 7 to 36 of joining for ewes grazing lucerne pasture, a period within which most ewes would have been in the most sensitive stages of early pregnancy (Parr, 1992).

The large difference in fetal numbers for ewes grazing lucerne compared with ewes grazing the senescent pasture is unlikely to be due to the observed weight loss between Day -7 and 7 for those in the senescent pasture treatment. The apparent weight loss in the senescent pasture and the low and varying weight gains for ewes in the lucerne treatments over this time are probably explained by the use of unfasted weights and a degree of measurement error. The level of weight loss to Day 7 recorded in the senescent pasture treatment was not consistent with the 0.1 loss in ewe condition score, nor with the maintenance of condition between the start and end of the grazing period. Furthermore, the fecundity of ewes in the senescent pasture treatment (1.49 fetuses per pregnant ewe) is above the average reported for Merino ewes (Kleemann et al., 2006), making it unlikely that ovulation rate was suppressed in this treatment. High ovulation rates can be produced by ewes in fat condition when fed sub-maintenance diets (Allen and Lamming, 1961), 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., 2014). 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., 2014) may be partially explained by joining occurring three weeks later in the present study, with ovulation rate increasing towards the peak of the breeding season (Dun et al., 1960). The presumed 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.

Embryo mortality can increase if ewes are fed 50% of maintenance requirement (Abecia et al., 2006), although feeding 25% of maintenance requirements between days 2 and 14 after mating did not reduce either pregnancy rate or fetal numbers, compared with ewes fed at maintenance, in the study of Parr et al. (1987). Pregnancy rate was similar between ewes grazing lucerne or senescent pasture in the current study. The weight loss observed prior to Day 7 for ewes grazing senescent pasture is not consistent with estimated intake or ewe condition change, and is likely overestimated due to the use of unfasted weights, since the ewes maintained both weight from Day 7 of joining, and condition score was similar at the start and end of the grazing period. It is therefore unlikely that the advantage in fetal numbers from grazing lucerne was due to sub-maintenance nutrition causing high rates of embryo mortality in ewes grazing senescent pasture.

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 was 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 in the current study. 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 mortality 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 (Fernandez-Twinn et al., 2003; Kwong et al., 2000; 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.

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. This is consistent with the trend (Table 5) for staple strength to differ between treatments. Calculations using woolcheque (www.woolcheque.com.au) demonstrate that even if the staple strength for ewes grazing lucerne was 3 N/ktex lower, the potentially 0.3 kg higher fleece weight in comparison with ewes grazing dead pasture meant that the value of wool produced was $3 per ewe higher. 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.

Differing paddock sizes (stocking rate) during joining are unlikely to have influenced the results. Ewe-ram contact may be influenced by paddock size (Fowler, 1976), with larger paddocks resulting in less ewes being mated. The proportion of ewes raddled was similar between treatments in the current study, and at the level expected if all ewes in oestrus were mated (6% per day) during the first 14 days of joining. While pasture availability could be altered by differing paddock sizes and stocking rates over the longer term, the availability of quality live pasture is the important factor driving the responses in fetal numbers in this study; and the quality/quantity of available pasture in senescent paddocks was sufficient to maintain liveweight.

It is not clear whether the better pre-lambing condition score of ewes which had grazed lucerne contributed to lamb survival and growth; differences in ewe liveweight pre-lambing can be partly attributed to differing fetal numbers. The survival of lambs from birth to marking was not reduced by grazing lucerne, and the similarity of lamb marking weights indicate no adverse production effect on lambs due to treatments. The increase in number of lambs marked per ewe joined as a result of flushing can be expected to vary depending on the level of lamb survival. The proportion of twin lambs born is increased as a result of flushing, but although not the case in this study, the survival of twin lambs is often lower than that of singles and much lower (Alexander et al., 1980) than reported here. Large increases in the number of lambs marked may not result from the higher ovulation rates attained by flushing, where environment or management severely restrict the survival of twins.

Conclusion

Grazing naturally cycling ewes on lucerne pasture before and during joining 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, when joining during the natural breeding season. At this time, most ewes can be expected to mate in the first 14 days of joining since oestrous cycles should be occurring in all ewes. 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.

Acknowledgements

The funding of Meat & Livestock Australia is gratefully acknowledged. The Australian Wool Education Trust also part funded this study. The study was made possible through access to sheep and pastures provided by the Robertson family. Students Laura Kemmis, Emma Knight and Emmanuelle Haslin assisted with some of the field work.

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

	Paddock	Plant type	Day-8	Day 6	Day 13	Day 27
ME (MJ/kg DM)	Lucerne	Live lucerne	11.3 ± 0.65	14.6 ± 0.20	12.6 ± 0.10	14.1 ± 0.05

	Senescent	Dead grass	5.8 ± 0.25	5.4 ± 0.15	5.3 ± 0.15	5.4 ± 0.25
	All paddocks	Live grass	12.7 ± 0.50 (combined days)			
CP (% of DM)	Lucerne	Live lucerne	19.9 ± 1.15	32.6 ± 0.65	32.9 ± 2.60	31.8 ± 1.70
	Senescent	Dead grass	2.2 ± 0.20	<2.0	<2.0	2.7 ± 0.65
	All paddocks	Live grass	27.4 ± 2.53 (combined days)			
NDF (% of DM)	Lucerne	Live lucerne	37.0 ± 2.00	27.5 ± 0.50	30.5 ± 0.50	30.5 ± 1.50
	Senescent	Dead grass	77 ± 0.50	81 ± 1.00	80 ± 2.00	79 ± 2.00
	All paddocks	Live grass	52.6 ± 2.25 (combined days)			
WSC (% of DM)	Lucerne	Live lucerne	3.4 ± 0.45	3.4 ± 0.15	2.5 ± 0.15	7.6 ± 1.53
	Senescent	Dead grass	1.2 ± 0.19	2.3 ± 1.29	2.1 ± 1.29	2.0 ± 0.98
	All paddocks	Live grass	4.9 ± 0.81 (combined days)			

Table 2 Estimated daily intake of metabolisable energy (MJ ME) and protein predicted by GrazFeed for ewes grazing senescent 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
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

Table 3 Mean (± sem) reproductive performance of ewes grazing senescent pasture (Days -7 to 36), lucerne throughout (Days -7 to 36) or lucerne to Day 7 (Days -7 to 7) of joining. Days relative to the introduction of rams.

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	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 ± 0.095 a	1.61 ± 0.095 b	1.60 ± 0.095 b	0.002
Per pregnant ewe	1.49 ± 0.081 a	1.71 ± 0.080 b	1.76 ± 0.081 b	<0.001

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

Table 4 Mean (± sem) production of lambs born to ewes grazing senescent pasture (Days -7 to 36), lucerne throughout (Days -7 to 36) or lucerne to Day 7 (Days -7 to 7) of joining. Days relative to the introduction of rams. . **Number of ewes or lambs in brackets.**

	Senescent pasture	Lucerne throughout	Lucerne to Day 7	P-value
Lambs born per ewe joined [A]	1.30 ± 0.060 a (127/98)	1.62 ± 0.06 b (162/100)	1.62 ± 0.060 b (160/99)	<0.001
Lambs born per ewe joined (Observed) [B]	1.25 ± 0.075 a (122/98)	1.55 ± 0.075 b (155/100)	1.57 ± 0.075 b (155/99)	0.003
Proportion lamb survival [C]	0.78 (95/122)	0.74 (114/155)	0.74 (115/155)	0.685
No. lambs marked per ewe joined	0.96 ± 0.060 a (94/98)	1.14 ± 0.060 b (114/100)	1.15 ± 0.060 b (114/99)	0.040
Dead weight of twins (kg)	5.1 ± 0.55 (19)	4.7 ± 0.52 (28)	4.7 ± 0.53 (23)	0.977

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

[A]Lambs born is calculated from scanned fetal number where actual lambs born was not observed (ewe didn't lamb or abandoned lambs before tagging); [B]Lambs born is calculated from observations during lambing; [C]Lamb survival calculated from observations during lambing, and excludes12 lambs born which could not be identified to ewes and therefore treatment.

Table 5 Mean (± sem) wool characteristics of ewes grazing senescent pasture (Days -7 to 36), lucerne throughout (Days -7 to 36) or lucerne to Day 7 (Days -7 to 7) of joining. Days relative to the introduction of rams.

	Senescent pasture	Lucerne throughout	Lucerne to Day 7	P - value
Greasy fleece weight (kg)	4.7 ± 0.11 n=93 n=20	5.1 ± 0.11 n=88 n=20	4.8 ± 0.11 n=93 n=20	0.057
Yield (%)	76 ± 0.73	74 ± 0.73	74 ± 0.73	0.099
Fibre diameter (microns)	19.6 ± 0.55	20.1 ± 0.55	19.2 ± 0.55	0.156
Staple length (mm)	74 ± 1.5	71 ± 1.5	72 ± 1.5	0.377
Staple strength (N/ktex)	34.0 ± 1.13	30.3 ± 1.13	31.2 ± 1.13	0.066
Minimum along-staple fibre diameter (microns)	18.0 ± 0.44	18.1 ± 0.44	17.5 ± 0.44	0.468
Maximum along-staple fibre diameter (microns)	21.2 ± 0.60	22.4 ± 0.60	21.4 ± 0.60	0.087
Coefficient of variation in fibre diameter along staples (%)	4.9 ± 0.38 a	6.6 ± 0.38 b	6.1 ± 0.38 b	0.001

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

Captions to Figures

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

Fig. 2. Mean a) unfasted live weight (kg ± sem) and b) condition score (score ± sem) of ewes in three treatments prior to and following the introduction of rams (Day 0). Symbols: senescent pasture (✹), lucerne throughout (■), lucerne to day 7 (□).

Fig. 3. Mean (± 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. Symbols: senescent pasture (✹), lucerne throughout (■), lucerne to day 7 (□). Means are backtransformed.