Flushing unsynchronised Merino ewes with lucerne for different time periods relative to joining does not affect embryo survival

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Abstract

It was investigated whether lucerne pasture could increase the ovulation rate and subsequent foetal number of unsynchronised ewes joined for 5 weeks in autumn, compared to ewes grazing senescent pasture, and whether embryo survival was affected. The effect of grazing lucerne on plasma P_4 and urea (measured on days 3, 7, and 14), and glucose concentrations (day 14) was investigated (n=48 ewes). Non-maiden Merino ewes (n = 300), 3.5 or 6 years of age were divided between three dietary treatments (two replicates of each): (1) grazing lucerne pasture from day -7 until day 7 of joining then grazing senescent pasture (Lucerne-14); (2) grazing lucerne pasture from day -7 until the end of joining (Lucerne-43); and (3) grazing senescent pasture from day -7 until the end of joining (Control). Ovulation rate and foetal number was determined for n=103 and 297 ewes, respectively. Estimated embryo mortality was calculated as the difference between foetal number and ovulation rate.

Grazing lucerne for either time period resulted in a 22% increase ($P<0.05$) in the number of ewes with multiple foetuses, without affecting estimated embryo mortality, the number of ewes returning to service, or the number of non-pregnant ewes ($P>0.05$). Mean plasma P_4 concentration was higher ($P<0.001$) in the Control group than the Lucerne-14 and Lucerne-43 groups. Plasma urea concentration was elevated ($P<0.001$) in the Lucerne-43 group compared

to the Control group. Blood glucose concentration was higher (P<0.001) in the Lucerne-43 group than the other groups. It was concluded that short-term grazing of lucerne could increase foetal number compared to ewes grazing senescent pasture. There seems to be little benefit to grazing lucerne for the entire joining period, as there was no increase in foetal number. Grazing lucerne does not appear to affect embryo survival.

Introduction

Ovulation rate is the main source of variation in the reproductive rate of ewes (Hanrahan 2003) and is readily affected by nutrition (Smith 1988; Viñoles *et al.* 2005; Robinson *et al.* 2006), providing a means through which producers can potentially improve their flock reproductive capacity. 'Flushing' is the practice of giving ewes high quality feed (which is high in energy and/or protein) for a short period prior to mating, to increase ovulation rates (Pearse *et al.* 1994); the exact time period varies, although most often supplementary feeding continues for 2-3 weeks (Sormunen-Cristian and Jauhiainen 2002). To date, the majority of flushing studies have used synchronised ewes, so the effectiveness of flushing naturally joined ewes is unclear. The most economical and effective feed type(s), and the optimum feeding period must also be identified.

It appears that increased dietary energy intake is the main driver for changes in ovulation rate, although high protein also contributes to the ovulatory response (Davis *et al.* 1981; Fletcher 1981; Ocak *et al.* 2006). Lupins (*Lupinus angustifolius*) have been frequently used in flushing studies, but the labour involved in feeding on a commercial scale may be a disadvantage. Other high energy feeds such as high quality green forage may provide a relatively inexpensive alternative.

Several green forages have already been used successfully in synchronised ewes, including *Lotus corniculatus* (Ramírez-Restrepo *et al*. 2005; Viñoles *et al*. 2009), tagasaste (*Chamaecytisus palmensis*; Wilkins 1997), chicory (*Chicorum intybus*) and lucerne (*Medicago sativa*; King *et al*. 2010); lucerne has also been used to flush unsynchronised ewes (Robertson *et al.* 2013). Of these, lucerne appears the most suitable for Australian conditions, providing high quality feed when most other pastures become senescent and lose their nutritional value (Thomas *et al.* 2010), and it has many additional benefits that extend to the rest of the enterprise (Humphries 2012). Where lucerne has been reported to reduce ovulation rates, this

occurred due to the presence of coumestans in the pasture (Kelly *et al.* 1976; Smith *et al*. 1979). This can be avoided by not grazing lucerne when the risk of increased coumestans is high.

Due to the conflicting nutritional requirements of the follicle and embryo (Robinson *et al.* 2002), managing the nutrition of multiple ewes at different stages oestrus and early pregnancy to ensure optimum ovulation rate and embryo survival will be complex. High feed intake on days 11 and 12 of pregnancy has been linked to a decline in circulating P_4 and subsequent pregnancy rate (Parr *et al.* 1987). High plasma urea is also thought to increase embryo loss (Bishonga *et al*. 1996; McEvoy *et al.* 1997; Berardinelli *et al*. 2001), and it has been recommended to avoid grazing ruminants on high-nitrogen, lush pastures during early pregnancy (Robinson *et al.* 2006). Minimising the flushing period to limit high energy and nitrogen intake during early pregnancy may be important to reduce embryo losses in flushed ewes.

Methods of flushing which involve only a short period of supplementation are also highly desirable from an economical and practical perspective, as it substantially reduces the quantity of feed required. Short-term feeding to achieve a significant flushing response is possible due to the acute effect of nutrition, which involves an ovulatory response without affecting liveweight (Knight *et al*. 1975; Scaramuzzi *et al.* 2006). This acute effect seems to be partially mediated by glucose (Venter and Greyling 1994; Iglesias *et al.* 1996; Downing and Scaramuzzi 1997), which acts directly on the ovary during short-term dietary supplementation (Viñoles *et al.* 2005).

Short-term flushing of ewes with lucerne has shown variable results, largely attributed to the inconsistent availability and quality of lucerne throughout the mating period and the presence of live pasture in the control treatments (King *et al.* 2010; Robertson *et al.* 2013). When 1300 kg DM/ha of lucerne was available, grazing unsynchronised ewes on lucerne for a week prior to and into the joining period produced an additional 18% foetuses, compared with ewes grazing cereal crop stubble (Robertson *et al.* 2013). Furthermore, there was no evidence of increased embryo mortality occurring in ewes grazing lucerne.

A flushing response has also been recorded in synchronised ewes grazing lucerne for 9 days prior to ovulation where only 350 kg DM/ha was available (King *et al.* 2010) and Robertson *et*

al. (2013) suggest that a minimum biomass of 100 kg DM/ha may be sufficient to flush unsynchronised ewes. Further investigation is required to determine the optimum pasture biomass and quality requirements to flush unsynchronised ewes.

The aim of this study was to firstly determine whether lucerne pasture could be used to flush naturally joined Merino ewes compared to ewes grazing senescent pasture in a commercial setting; and whether grazing lucerne for longer than 7 days into joining would affect estimated embryo mortality. Some of the potential mechanisms involved in flushing and embryo mortality were also investigated, by measuring blood P_4, urea and glucose concentrations; however, determining the actual processes was beyond the scope of this study.

Materials and Methods

This study was conducted on a property located 6.5 km northeast of Marrar in Southern NSW (34'48°S, 147'26°E) from 28 February to 3 June 2014. The average minimum/maximum daily temperatures during the experimental period varied from 18/33°C in February to 5/14°C in June, measured at the Wagga Wagga meteorological station 38 km from Marrar. Approval had been granted by the Charles Sturt University's Animal Care and Ethics committee (protocol number 13/088) prior to the commencement of this study.

Animals and experimental procedures

The study involved 300 medium framed (54.1 ± 0.3 kg), non-maiden Merino ewes, either 3.5 or 6 years of age sourced from a commercial flock on the Marrar property. There were three dietary treatments, with two replicates of each. The three treatments were: (1) grazing lucerne pasture from day -7 until day 7 of joining then grazing senescent pasture (Lucerne-14); (2) grazing lucerne pasture from day -7 until the end of joining (Lucerne-43); and (3) grazing senescent pasture from day -7 until the end of joining (Control).

The ewes were separated into age cohorts and randomly allocated to each treatment (n=100, 50 ewes from each age group). All of the ewes were weighed (Gallagher Rudweigh™ weigh scales) and assessed for body condition score (BCS) without fasting, a week prior to joining (day -7), and at fortnightly intervals thereafter until the end of the joining period (day 36). All treatment groups were introduced to their respective pastures on day -7. The Lucerne-14

ewes grazed with the Lucerne-43 group until day 7, after which they (Lucerne-14) were moved to the senescent pasture to graze with the Control group.

Merino rams were introduced to the ewes on day 0, with two rams per group of 100 ewes. Non-experimental ewes were included in the flocks to maintain equal numbers of ewes per ram, before and after the Lucerne-14 ewes had been moved to senescent pasture. Each ram was harnessed with a crayon marker (raddle), and marks were recorded on days 3, 7, 12, 14, 21, and 28. On the same days that raddled marks were recorded, the rams were rotated between groups and partners to avoid ram bias. The crayon colour was changed on day 14, so that it could be determined whether the ewes became pregnant in the first 14 days (similar to the first oestrous cycle). The timeline of the main procedures is shown in Figure 1.

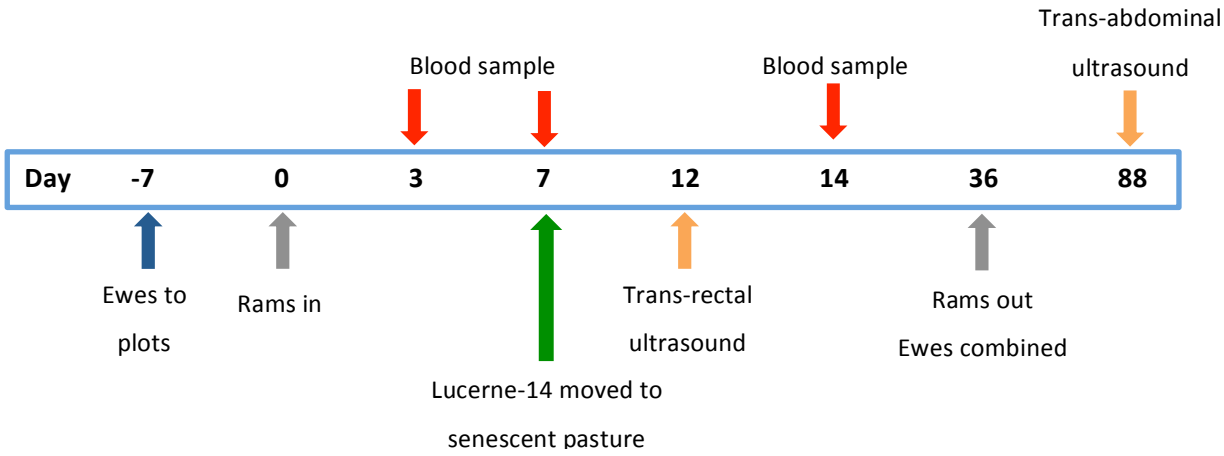

Figure 1 Timeline of main procedures.

Blood samples were collected from the jugular vein (BD Vacutainer® Heparin; 18 gauge needle) from a total of 48 ewes (16 from each treatment) on the morning of days 3, 7, and 14. These ewes had been mated between days 0-3 (as identified by raddle) and were therefore at a similar stage of oestrus. For collection days 3 and 7 the samples were stored on ice prior to being centrifuged (3000 rpm x 15 minutes) and the resulting plasma and haemocytes were stored frozen until subsequent analysis of P_4 and urea concentrations. For the day 14 blood samples, the glucose concentration of the whole blood sample was determined (Accu-Chek® Performa) immediately after collection; the samples were then stored on ice and processed as above. Only plasma samples for n=12, 14, and 14 ewes from Control, Lucerne-14 and Lucerne-43, respectively, were analysed for day 7 plasma urea concentrations, as some plasma samples were misplaced prior to analysis.

Ovulation rate (the number of corpora lutea per ewe ovulating) was measured by trans-rectal ultrasonography on day 12 of joining, in 103 ewes that had been raddled by this day (n=31, 35, 37 from Lucerne-14, Lucerne-43, and Control groups, respectively). All ewes chosen were shown to have ovulated. At the conclusion of the dietary treatments (day 36), rams were removed and the ewes were combined as one flock. Foetal number was determined by trans-abdominal ultrasound on day 88.

Pastures

Three paddocks were available for the senescent pasture treatment. In these paddocks the available feed was predominantly senescent phalaris (*Phalaris aquatica*) and some actively growing weeds, which were mainly witch grass (*Panicum capillare*). Five lucerne pasture paddocks were used and primarily contained dryland lucerne and some weeds as for the senescent pasture. One of the lucerne paddocks contained lucerne as well as lupin stubble. The senescent pasture paddocks were sprayed with herbicide (glyphosate) on 8 and 23 March to remove the fresh green forage that grew in response to recent rainfall. Paddock sizes ranged from 20 to 36 ha for the lucerne pasture paddocks, and 7 to 20 ha for the senescent pasture paddocks.

Replicate two of the Lucerne-14 and Lucerne-43 groups were moved to a fresh lucerne paddock on days -2 and 3 when the proportion of lucerne leaf was visually estimated to have fallen below 50%. Replicate two of the Lucerne-43 group was moved again on day 21. Replicate two of the Control group was moved to a different senescent pasture paddock on day 3 when live pasture grew in the original paddock. Replicate one of the Lucerne-43 and Control groups remained in the same paddock throughout the joining period, and replicate one of the Lucerne-14 group moved between these paddocks.

Live and dead pasture biomass was visually estimated the day the rams were introduced to the ewes (day 0), and on days 6, 13, 20, 27, and 34 thereafter, using the method of Haydock and Shaw (1975) as described by Cayley and Bird (1996), using circular calibration quadrats ($0.25m^2$) cut at ground level with hand shears. Sixty visual estimates were recorded on all occasions in each paddock. Where ewes had been moved to another paddock, pasture biomass was estimated in both the original and new paddock. Except for Day 0, lucerne plant height was also measured on the same days biomass was estimated.

Samples of live lucerne from each of the lucerne paddocks and dead grass in each of the senescent pasture paddocks were taken using the toe-cut method (Cayley and Bird 1996) on Days -8, 6, 13 and 27, to assess pasture quality. All sub-samples of a single pasture type from each paddock were combined and analysed for metabolisable energy (ME), dry matter digestibility (DMD), and crude protein (CP) content by the Feed Quality Service of NSW Department of Primary Industries (Wagga Wagga, NSW). Pasture samples were ground through a 1 mm screen. Proximate analysis was determined by near-infrared spectroscopy with a Bruker multi-purpose analyser (Bruker Optik GmbH, Ettlingen, Germany) and calibration curves as described by Packer *et al.* (2011). The proportion of live lucerne stem and leaf was measured from the toe-cut lucerne samples by stripping leaves from the stem, drying and weighing.

Pluck samples of live witch grass and annual grasses were collected by selectively picking live pasture (avoiding senescent pasture) from 30 circular quadrats placed at random throughout the pasture. These samples were taken on Days 13 and 27 from the lucerne pasture paddocks, and on Days 6, 13, and 27 from the senescent pasture. The pluck samples were analysed as for the toe-cut samples.

Statistical analyses

All analyses were completed using ASReml-R (Butler *et al.* 2007; R Development Core Team 2010). A linear mixed model using restricted maximum likelihood was used to analyse the data for liveweight, BCS, plasma P_4 concentration, plasma urea concentration, blood glucose concentration, pasture biomass and pasture quality (Table 1). For analysis of variance (ANOVA) and weighted least squares analyses, all model assumptions were met. Where heterogeneity existed, data were transformed using natural logs (Table 1). Foetal number was included in each model initially and was removed, as it was not significant. Simple correlations between foetal number and plasma P_4/urea/glucose concentrations or ewe age were calculated using Microsoft Excel (2011). All reported values are predicted means ± standard error of the mean (SEM), unless otherwise specified. Raw means ± SEM were calculated using Microsoft Excel (2011) for descriptive purposes.

Table 1 Models used for linear mixed modelling analyses

Response variable	Analysis	Model
Day -7 liveweight	ANOVA	Liveweight ~ *rep* + trt + age + trt:age
Liveweight change	WLSA[A]	Liveweight change ~ *rep* + trt + age + trt:age
Day -7 BCS	ANOVA	BCS ~ *rep* + trt + age + trt:age
BCS change	WLSA	BCS change ~ *rep* + trt + age + trt:age
Plasma progesterone	WLSA	ln(progesterone) ~ *rep* + trt + day + age + foetal number + day:trt + trt:age + day:age + day:trt:age
Plasma urea	WLSA	ln(urea) ~ *rep* + trt + day + age + foetal number + day:trt + trt:age + day:age + day:trt:age
Blood glucose	ANOVA	Glucose ~ *rep* + trt + age + foetal number + trt:age
Pasture ME	WLSA	ME ~ day + pasture type + *paddock*
Pasture CP	WLSA	CP ~ day + pasture type + *paddock*
Pasture DMD	WLSA	DMD ~ day + pasture type + *paddock*
Pasture live biomass	WLSA	ln(live biomass + 1) ~ day + pasture type + day:pasture type + *pasture type:paddock*
Pasture dead biomass	WLSA	ln(dead biomass + 1) ~ day + pasture type + day:pasture type + *pasture type:paddock*

[A] Weighted least squares analyses; trt = treatment; rep = replicate; age = ewe age; day = experimental day. Random effects are italicised.

A generalised linear model with a Chi-Squared test for independence using Fisher's exact test was used to analyse the proportion of ewes with multiple ovulations, ewes with multiple foetuses, ewes raddled twice (i.e. ewes returning to service), ewes raddled in the first cycle, and non-pregnant ewes. Ewe age was fitted in each model, although was not significant and so was excluded from the final model. As the number of ovulations was 1, 2, or 3, and there was only one triplet ovulation, the data were converted into two factors (single and multiple ovulations) and analysed as a binomial trait. The proportion of ewes with multiple foetuses was calculated for pregnant ewes only, and foetal number was also converted into a binomial trait as for ovulation rate. The final model can be written symbolically as:

Response ~ treatment

For the ewes selected for trans-rectal ultrasonography, estimated embryo mortality was calculated as the difference between foetal number and the number of ovulations for each individual ewe (assuming no fertilisation failure), and converted into a factor (no embryo loss, partial embryo loss, or total embryo loss). Partial loss refers to those ewes that have lost some (≥1), but not all embryos. Where foetal number was greater than ovulation rate, ovulation rate was adjusted to equal foetal number. The proportion of ewes with total, partial, and no embryo loss was analysed using a Fisher's exact test, as for the proportion of ewes with multiple ovulations. Ewe age was not significant so was not included in the final model.

One ewe from the Control group was euthanised due to illness so all data for this ewe were excluded from analysis. Three ewes were not present at the first weighing (Control n=1; Lucerne-14 n=2) and two were missing at final weighing (Control n=1; Lucerne-43 n=1); these data were not included in the weight change or BCS change analysis. The blood glucose concentration for one ewe (Lucerne-14) was considered erroneous and was excluded from analysis. At pregnancy scanning, two ewes from the Lucerne-43 group were absent; one ewe from the Lucerne-43 group had been submitted for trans-rectal ultrasonography and so its data on foetal number was not available for calculating embryo mortality. Where raddle marks were very faint or questionable, they were not included in the analysis of ewes raddled in the first cycle (Control n=1), or ewes raddled twice (Control n=3; Lucerne-14 n=6).

Results

Liveweight and body condition score

At the commencement of the trial the liveweight (Figure 2) and BCS (Table 2) of the ewes was similar ($P>0.05$) across the treatment groups, although the mean BCS differed ($P \leq 0.05$) between age groups (2.8 ± 0.04 and 2.9 ± 0.04 for 3.5 and 6 year old ewes, respectively). By the end of the dietary treatments (day 36) the average liveweight ($P<0.001$) and BCS ($P<0.001$) of the ewes varied between the treatment groups: Lucerne-43 ewes had gained weight whilst both the Lucerne-14 and Control ewes lost weight throughout the joining period (Table 2). Both Lucerne groups gained body condition, while the mean BCS for the Control declined over the joining period (Table 2). Ewe age had no effect ($P>0.05$) on final BCS.

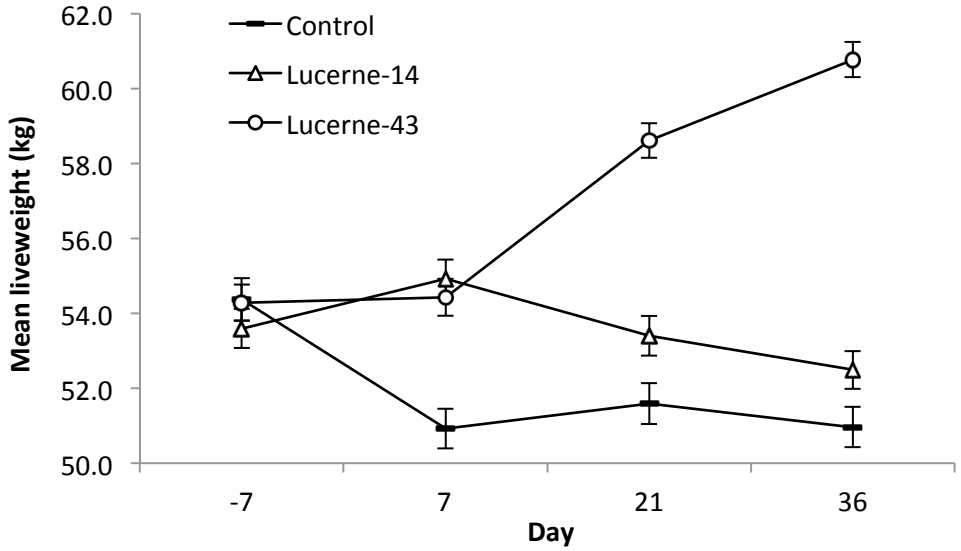

Figure 2. Liveweight change of ewes over the joining period (values are raw means ± SEM).

Table 2 Body condition score of the ewes on day -7 and their BCS and liveweight change over the joining period

Treatment	Control	Lucerne-14	Lucerne-43	P-value
BCS (day -7)	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	0.998
Total change in BCS	-0.1 ± 0.0[a]	0.1 ± 0.0[b]	0.8 ± 0.0[c]	<0.001
Total liveweight change (kg)	- 3.4 ± 1.2[a]	- 1.0 ± 1.2[b]	+ 6.5 ± 1.2[c]	<0.001

Values within rows with different superscripts vary significantly.

Reproductive performance

As shown in Table 3, subjecting ewes to different dietary regimens during the joining period significantly affected the proportion of ewes with multiple ovulations ($P<0.05$) and the proportion of pregnant ewes carrying multiple foetuses ($P<0.01$). The majority of multiple ovulations were twins, with only one triplet ovulation recorded in the Lucerne-43 group. Only four triplet pregnancies were recorded, all in the Lucerne-14 group. For each treatment, a similar ($P>0.05$) number of ewes were raddled in the first 14 days, and recorded as non-pregnant at pregnancy scanning.

Table 3 Reproductive performance of ewes in each treatment group

Treatment	Control	Lucerne-14	Lucerne-43	P-value
Mean ovulations/ewe[A]	1.62 ± 0.08	1.71 ± 0.08	1.91 ± 0.06	-
Ewes with multiple ovulations (%)	62[a]	71[ab]	89[b]	0.032
Mean foetuses/ewe[A]	1.49 ± 0.07	1.75 ± 0.07	1.71 ± 0.06	-
Ewes with multiple foetuses[B] (%)	0.49[a]	0.71[b]	0.71[b]	0.003
Ewes raddled by day 14 (%)	88	83	92	0.163
Non-pregnant ewes (%)	12	9	6	0.333

Values within rows with different superscripts vary significantly. [A] Values are raw means ± SEM. [B] Excludes non-pregnant ewes.

Estimated embryo mortality

The effect of dietary treatments during joining on embryo loss and (ewe) return to service is shown in Table 4. Subjecting ewes to different dietary regimens during the joining period had no effect (P>0.05) on the incidence of total or partial embryo losses. In addition, the percentage of ewes with no embryo loss did not differ (P>0.05) between treatment groups. The percentage of ewes that returned to service also did not differ (P>0.05) between treatment groups.

Table 4 Embryo loss and returns to service across the treatment groups

Treatment	Control	Lucerne-14	Lucerne-43	P-value
No embryo loss (%)	65	61	68	0.915
Partial embryo loss (%)	27	26	26	0.915
Total embryo loss (%)	8	13	6	0.915
Returns to service [A] (%)	22	17	20	0.535

[A] Excludes non-pregnant ewes

Plasma progesterone concentration

Mean plasma P_4 concentration (across all three sampling days) for ewes in the Control group were higher ($P<0.001$) than for the Lucerne-14 and Lucerne-43 ewes, which were similar to one another (Figure 3). Concentrations of plasma P_4 increased ($P<0.001$) with time independently of treatment. Foetal number did not affect mean plasma P_4 concentration ($P>0.05$). There was no correlation between plasma P_4 concentration and foetal number ($R^2 = 0.006$) or ewe age ($R^2 = 0.01$).

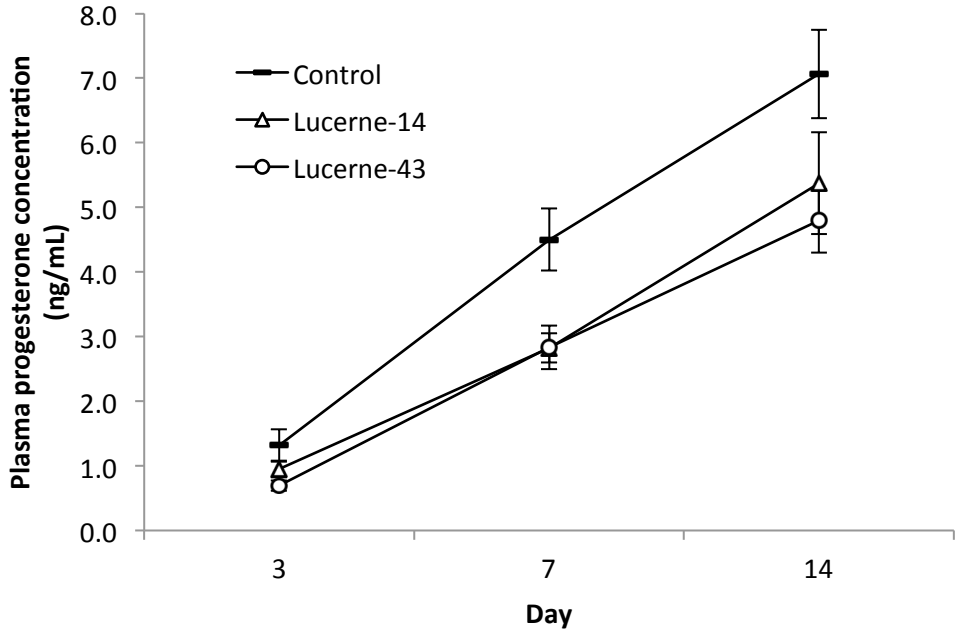

Figure 3. Plasma progesterone concentrations on days 3, 7, and 14 for each treatment (values are raw means ± SEM).

Plasma urea concentration

On each sampling day, concentrations of plasma urea were higher ($P<0.001$) in the Lucerne-43 ewes than in the Control ewes (Figure 4). However, within the Lucerne-43 and Control groups the plasma urea concentration did not vary ($P>0.05$) between sampling days. Lucerne-14 plasma urea concentrations were similar to Lucerne-43 on days 3 and 7, declining ($P<0.001$) on day 14 to become similar to that of the Control (Figure 4). There was an interaction between day and treatment ($P<0.001$). Foetal number did not affect plasma urea concentration ($P>0.05$). There was no correlation between plasma urea concentration and foetal number ($R^2 = 0.003$) or ewe age ($R^2 = 0.09$).

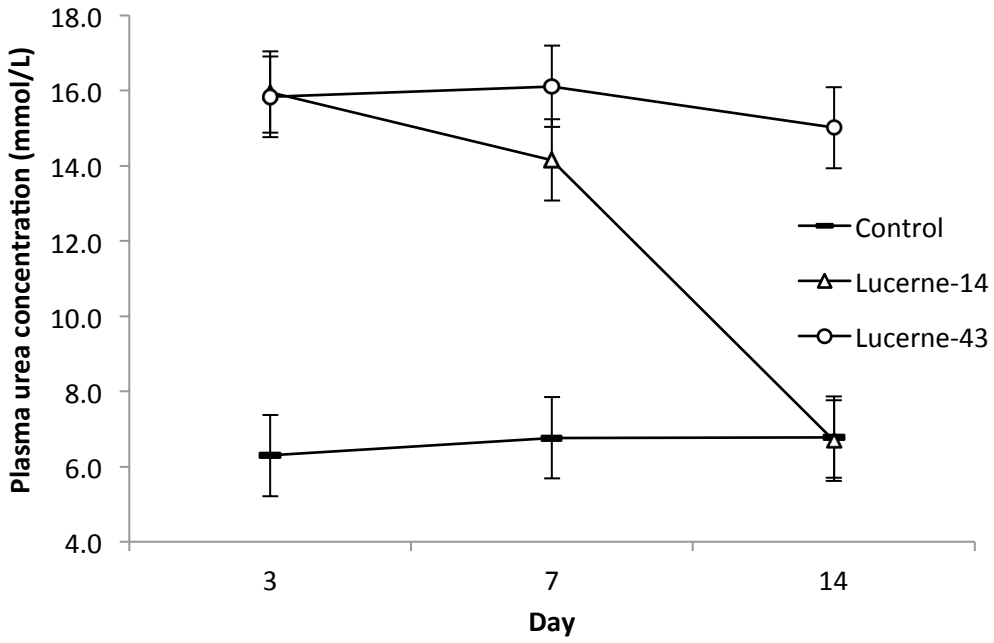

Figure 4. Plasma urea concentrations on days 3, 7, and 14 for each treatment.

Blood glucose concentration

Blood glucose concentrations (on day 14) were significantly higher ($P<0.001$) in the Lucerne-43 ewes (4.9 ± 0.1 mmol/L) compared to the Lucerne-14 (4.0 ± 0.1 mmol/L) and Control (4.0 ± 0.1 mmol/L) ewes. Foetal number did not affect blood glucose concentration ($P>0.05$). There was no correlation between blood glucose concentration and foetal number ($R^2 = 0.003$) or ewe age ($R^2 = 0.02$).

Pasture biomass and composition

As shown in Figure 5, a greater ($P<0.001$) amount of live forage (lucerne plus other live forage) was available in the lucerne pasture compared to the senescent pasture on days 0, 13, and 34 of the joining period. The amount of live forage in both the lucerne and senescent pastures increased ($P<0.001$) throughout the joining period. There was an interaction between pasture type and day ($P<0.001$).

The senescent pasture contained a greater ($P<0.001$) quantity of dead forage throughout joining period compared to the lucerne pasture. The amount of dead forage in the lucerne

pasture increased (P<0.001) from day 0 of joining (Figure 5) due to changes in paddock. Changes in dead forage biomass in the senescent pasture were not significant (P>0.05). For dead forage biomass, day was not significant (P>0.05), although there was an interaction between day and pasture type (P<0.001). On days 0, 13, and 34, lucerne contributed to 93, 96, and 70% of total live forage in the lucerne pasture, respectively.

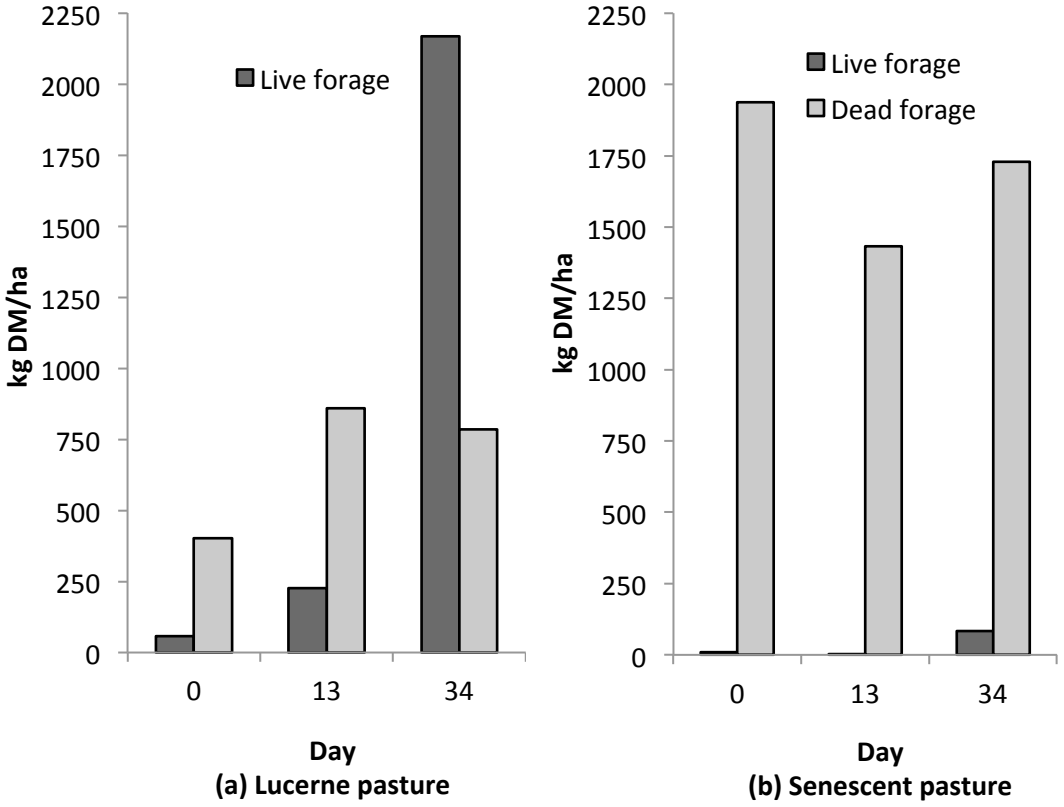

Figure 5. Mean quantity and composition of pasture available in the (a) lucerne and (b) senescent pasture paddocks on days 0, 13, and 34 of joining.

Pasture quality, proportion of lucerne leaf and lucerne height

The nutritive value (ME, CP and DMD) of the lucerne was much higher (P<0.001) than that of the dead forage (Tabl5). Pasture quality did not vary (P>0.05) over the joining period. The proportion of lucerne leaf varied from 48 to 64%, with mean plant height ranging from 21.99 ± 0.86 to 30.78 ± 1.20 cm. The nutritive value of witch grass and annual grass (combined for the lucerne and senescent pastures) is shown in Table 6.

Table 5 Mean ME, CP, and DMD of live lucerne in the lucerne pasture, and dead grass in the senescent pasture

Pasture type	ME (MJ/kg DM)	CP (%)	DMD (%)
Lucerne	13.1 ± 0.5^{b}	29.1 ± 2.5^{b}	85.9 ± 2.9^{b}
Dead forage	5.4 ± 0.1^{a}	2.2 ± 0.2^{a}	41.1 ± 0.7^{a}

Values within columns with different superscripts vary significantly ($P<0.001$).

Table 6 Mean ME, CP, and DMD of witch grass and annual grass from the lucerne and senescent pastures (Values are raw means ± SEM)

Pasture type	ME (MJ/kg DM)	CP (%)	DMD (%)
Witch grass	12.4 ± 0.8	24.8 ± 4.9	81.5 ± 4.5
Annual grass	12.9 ± 0.8	29.1 ± 3.1	84.3 ± 4.3

Discussion

Grazing lucerne pasture for 14 days, beginning 7 days prior to joining, was sufficient to flush naturally joined Merino ewes by increasing the number of ewes with multiple foetuses, compared to ewes grazing senescent pasture. Grazing lucerne for 7 days prior to and throughout the joining period increased the number of ewes with multiple ovulations as well as foetuses relative to ewes grazing senescent pasture; however, the flushing response was not greater than that achieved by the shorter grazing period, so there appears to be little benefit to grazing lucerne beyond day 7 of joining. In addition, grazing lucerne for either time period had no apparent effect on estimated embryo mortality, as there were no differences between the dietary treatments for total, partial, or no embryo loss, nor ewes returning to service.

Flushing response

A significant increase in foetal number was achieved from short-term grazing of lucerne, which could therefore substantially reduce the quantity of feed required to flush unsynchronised ewes, allowing producers to use the remaining lucerne for alternative purposes. The 22%

increase in pregnant ewes with multiple foetuses in the Lucerne groups was greater than the corresponding 16% increase in one of the experiments reported by Robertson *et al.* (2013), which involved flushing unsynchronised Merino ewes on lucerne for 7 days prior to and after the start of joining, with control ewes grazing cereal crop stubble. The ovulatory response in this study was also far greater than that found by King *et al.* (2010) who flushed synchronised ewes using lucerne. The greater flushing response recorded in the present study compared with previous studies may be related to the consistently higher quality and availability of lucerne in this study. The increased number of ewes with multiple foetuses in this study was also higher than that reported for unsynchronised ewes fed lupins; Lightfoot *et al.* (1976) were only able to achieve a 10.6% increase in twinning ewes (out of pregnant ewes) from 25 days of lupin supplementation, beginning 7 days prior to the start of a natural joining.

It is likely that there was no further increase in the number of ewes with multiple foetuses in the Lucerne-43 group as 92% of ewes had already been mated by day 14 (effectively the first oestrous cycle; Table 4), so relatively few non-pregnant ewes remained to be flushed, even when accounting for the small number of ewes returning to service. The majority of multiple pregnancies were twins and this is also of benefit to producers, as triplet lambs have reduced rates of survival (Muir and Thomson 2009). Robertson *et al.* (2013) similarly found that the large majority of multiple foetuses resulting from flushing ewes with lucerne were twins.

The flushing response can be attributed to the greater amount of live feed (predominantly live lucerne) in the lucerne pasture compared to that in the senescent pasture throughout the joining period, as it is the total amount rather than the type of live pasture that causes the ovulatory response (King *et al.* 2010). Although less than 100 kg DM/ha of live feed was available in the lucerne pasture on day 0, the proportion of leaf and height of the lucerne meant that the majority of live feed was likely to be readily accessible to the ewes. Furthermore, sheep are capable of obtaining 80% of their diet from live plant material when there is at least 40 kg DM/ha available (Mulholland *et al.* 1976), so it is unlikely that ewe intake of live forage was limited. This is evidenced by the large weight gain of the Lucerne-43 ewes over the joining period. The smaller quantity of live feed that was available in the senescent pasture would have been consumed quickly, and despite the witch grass and annual grass having a similar nutritive value to the lucerne, live feed was evidently not present in sufficient quantities to maintain the weight of the Control ewes and Lucerne-14 ewes (once moved to

the senescent pasture); thus the live feed in the senescent pasture was unlikely to have been sufficient to increase ovulation rates in these ewes.

Blood glucose concentration

Many metabolites and hormones are suspected to regulate ovulation rate in response to dietary changes (Scaramuzzi *et al.* 2011), although there is evidence to suggest that glucose may mediate ovulation rate in response to short-term nutritional variation (Downing *et al.* 1995; Iglesias *et al.* 1996), because it is the main source of energy for the ovary (Rabiee *et al.* 1997; Viñoles 2003). As blood glucose concentrations were only measured on the one occasion (day 14), only limited conclusions can be drawn from the results. Blood glucose was influenced by the pasture type being consumed, and was elevated in ewes grazing lucerne presumably due to the higher ME content of the forage. It cannot be established whether glucose was related to the ovulatory response.

The normal range for blood glucose in the sheep is 2.78 to 4.44 mmol/L (Smith 2014), and blood glucose concentrations for both groups grazing senescent pasture (at the time of sampling) were within this range, with the blood glucose concentration of the Lucerne-43 group ewes being slightly above the maximum value. It would have been useful to measure blood glucose concentrations on a number of days when both Lucerne groups were grazing lucerne, and after Lucerne-14 had been moved to the senescent pasture, to monitor the changes in blood glucose and to develop an association between plasma glucose concentrations, pasture type, and the flushing response.

Estimated embryo mortality

Estimated embryo mortality was not significantly affected by dietary treatment; however, the 20% decrease in foetal number compared with the number of estimated ovulations for the Lucerne-43 group requires further consideration, as it could potentially indicate a greater degree of embryo loss not identified by the methods used to estimate embryo mortality. However, the most plausible reason for this difference relates to the degree of accuracy associated with trans-rectal ultrasound. The mean ovulation rate for each treatment, and particularly the Lucerne-43 group was much higher than the average (1.3) reported for the Merino breed (de Graaf 2010), so it may be that the operator mistook large pre-ovulatory

follicles for corpora lutea and thus overestimated ovulation rate in many of the ewes. In addition, flushed ewes have a greater number of enlarged follicles compared with non-flushed ewes (Rhind and McNeilly 1986; Xu *et al.* 1989; Viñoles *et al*. 2005) so it is possible that the operator overestimated ovulation rate for the Lucerne-43 group to a greater extent than for the other treatments as the Lucerne-43 ewes were still grazing lucerne on day 12, and thus follicles were still being affected by the flushing treatment. Furthermore, the accuracy of trans-rectal ultrasound for measuring ovulation rate is poor relative to methods such as laparoscopy (Dickie *et al.* 1999), increasing the difficulty of distinguishing between ovarian structures.

It is reasonable to conclude that although the estimates of embryo mortality in the present study were likely to have been overestimated, the relative comparison between treatments for embryo mortality is valid, and as such embryo mortality was not influenced by treatment. In support of this, the number of ewes returning to service did not vary between treatments, and Robertson *et al.* (2013) also found no evidence to suggest grazing lucerne increased embryo mortality in unsynchronised ewes. Therefore, it appears that grazing lucerne for longer than 7 days into joining does not pose a risk to embryo survival in naturally cycling ewes; this affords producers greater flexibility when flushing ewes, with the option to graze ewes on lucerne pasture for a longer period if it suits management practices, without the risk of increasing embryo loss.

Plasma progesterone concentration

It has been previously demonstrated that high feed intake increases plasma P_4 clearance, which has been associated with increased embryo mortality, particularly if high feed intake occurs on days 11 and 12 of pregnancy as the embryo is most susceptible to low P_4 concentration at this time (Parr *et al.* 1987; 1993). Parr *et al.* (1987) reported a 20% decrease in pregnancy rates of synchronised ewes consuming twice maintenance requirements, which also resulted in a decline in plasma P_4 concentration of 0.5 ng/mL**.** Brien *et al.* (1981) also found that supplementing unsynchronised non-maiden ewes with lupins (500 g/sheep.day) decreased plasma P_4 concentration by 0.6 ng/mL, which was thought to be responsible for the 7 and 18% increase in embryo loss compared to supplemented ewes consuming irrigated pasture or pasture hay, respectively.

Although feed intake was not measured in this study, it can be estimated that the Lucerne-43 ewes (and Lucerne-14 ewes while grazing lucerne pasture) could consume close to 1.9 x maintenance requirements and would gain 135 g liveweight/d based on the lucerne pasture characteristics and ewe liveweight (GrazFeed 4.1.13, Freer *et al.* 1997. Intake would not have been as high for the Control ewes due to the lower nutritive value and lower digestibility of the senescent pasture compared with the live lucerne. The lower digestibility would have decreased the rate of passage of feed through the rumen and thus decreased voluntary feed intake (Blaxter *et al.* 1961). The higher level of feed intake for the Lucerne-43 ewes is reflected in their higher liveweight and BCS compared to the Control ewes; Lucerne-43 ewes gained 151 g/d over the course of the experiment, higher than that indicated by GrazFeed, suggesting that they were consuming at least twice their maintenance requirements. Consistent with previous studies, mean plasma P_4 concentrations were significantly lower in the Lucerne groups than the Control group in this study, presumably due to increased feed intake; however, this does not seem to have affected embryo survival, perhaps as the approximate mean plasma P_4 concentrations for the Lucerne groups on days 11 and 12 were within or slightly above the optimal range of 2-4 ng/mL defined by Parr *et al.* (1987).

Plasma urea concentration

Based on findings in dairy cows, Robinson *et al.* (2006) have suggested that fresh pastures can negatively affect embryo survival due to their high nitrogen content. High dietary nitrogen intake (as either protein or urea) is suspected to reduce ovine embryo viability by raising plasma urea and/or ammonia concentrations (Bishonga *et al*. 1996; McEvoy *et al.* 1997; Berardinelli *et al*. 2001). However, the literature is inconsistent, with reports of increased embryo mortality as well as accelerated embryo development resulting from high protein dietary supplementation. Even less is known of the mechanisms involved. Berardinelli *et al*. (2001) proposed that a high plasma urea concentration in ewes causes the rate of embryo development to become asynchronous with the uterine environment; however, plasma urea concentration was not correlated with embryo loss itself. McEvoy *et al.* (1997) suggested that elevated plasma urea/ammonia caused embryo mortality, yet later development and metabolism was improved in those embryos that did survive.

Despite the ewes in the Lucerne-43 treatment having a significantly greater plasma urea concentration (15.83-16.11 mmol/L) compared with the Control (6.29-6.78 mmol/L) on all

three sampling days, and the plasma urea concentration for the Lucerne-14 group also being elevated on days 3 and 7 (14.15-15.95 mmol/L), there was no evidence of increased embryo mortality in either of the Lucerne groups. Bishonga *et al.* (1996) and McEvoy *et al.* (1997) recorded greater embryonic loss in ewes with plasma urea concentrations of 6-8 mmol/L and based on these values, increased embryonic death would be expected in all treatment groups in the present study, particularly in the Lucerne groups. It is plausible that embryo loss was not increased by the elevated plasma urea concentrations in the present study, due to the vastly different experimental conditions compared with previous studies (Bishonga *et al*. 1996; McEvoy *et al.* 1997; Berardinelli *et al*. 2001). In the previous studies, many ewes were synchronised, superovulated, and artificially inseminated; ewes were fed a urea supplement once or twice daily for up to 14 days before ovulation and 63 days into pregnancy, and fasted prior to embryo collection; embryos were also examined *in vitro* to determine their viability, and single embryos were returned to ewes to allow pregnancy to continue.

The number of invasive procedures and differing dietary conditions may have altered the sensitivity of the embryo to plasma concentrations of urea/ammonia, causing an effect that did not eventuate in the present study: a grazing situation, where ewes were allowed to graze a protein form of nitrogen continuously and largely undisturbed. The small number of ewes and embryos in many of the previous studies are also limiting factors, as individual ewe performance can greatly affect the mean of the treatment group. For example, Bishonga *et al.* (1996) found one ewe had superovulated unexpectedly, skewing the mean ovulation rate for that group which contained only 10 ewes.

Conclusion

Grazing ewes on lucerne pasture for a short period of only 14 days (commencing 7 days before joining) was sufficient to flush naturally joined ewes, significantly increasing the number of ewes with multiple foetuses, the majority of which were twins. Where ewes are naturally cycling and fertile, there seems to be little benefit to grazing lucerne for the entire joining period, as there was no further increase in foetal number compared with the shorter grazing period. Grazing lucerne beyond day 7 of joining did not appear to have a negative effect on the embryo, despite the significant differences in plasma P_4 and urea concentrations between ewes grazing lucerne and those grazing senescent pasture.

This latter finding suggests that the well-described mechanisms involving P_4 and embryo mortality in synchronised ewes may not hold true for a grazing situation with unsynchronised ewes. Also, it is clear that further research is needed to define the effects of high plasma urea concentration on embryo survival, as current literature is inconclusive. Naturally joined ewes were significantly flushed from short-term grazing of lucerne, which substantially reduced the quantity of feed required, demonstrating the benefit of using lucerne to flush commercial ewes.

It has now been demonstrated in several experiments that lucerne is of value as a flushing feed. Lucerne availability and quality limited the ovulatory response in some previous studies; nonetheless this study has illustrated that foetal number can be substantially increased when ample, high quality pasture is available. Future research will add to our understanding of the minimum biomass and quality required for flushing to be a success, which may lead to the development of guidelines for use by sheep producers. Additionally, the economic value of flushing ewes with lucerne needs to be evaluated; not only in relation to the costs involved in sowing and feeding the lucerne, but also in terms of lost revenue from alternative uses of the feed, such as finishing lambs. This will be important in order to encourage commercial producers to adopt the practice.

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