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Frequency of feeding during the periconceptional period did not alter reproduction in Merino sheep

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ABSTRACT

Context. During drought, it is currently recommended to feed complete rations to sheep every second or third day, rather than daily, to reduce labour costs and the incidence of shy feeders. However, the frequency of feeding to ewes in the periconceptual period may influence fertility and fecundity and therefore profitability. **Aims.** The study was designed to determine whether the feeding frequency of maintenance energy levels during joining affects reproductive performance and wool production of Merino ewes. **Methods.** A group-fed pen study was conducted using two treatments and four replicates. Naturally oestrous-cycling Merino ewes ($n = 800$) were fed barley grain (90%) and wheat straw (10%) from 17 days before ram introduction until Day 30 of a 48-day joining period, either once-daily or on alternate days at maintenance energy levels. Lambing performance was recorded for 505 ewes pregnant by Day 19 of joining. Wool quality was assessed on Day 117 after commencement of joining. **Key results.** Clinical acidosis occurred in both treatments, although the rate of mortality was low (4/800). The proportion of shy feeders was not affected ($P = 0.486$) by feeding frequency ($n = 77$, removed from pens). For the remaining ewes, weight loss was reduced by 1 kg ($P = 0.003$) when fed daily. Plasma progesterone concentrations post-mating were reduced ($P < 0.001$) by 18% with alternate-day feeding. Proportions of ewes mated, returning to service, pregnant or bearing multiple fetuses, lamb survival, lamb weights at marking and ram semen morphology were similar ($P > 0.05$) between feeding groups. Wool fibre diameter, staple strength and yield were similar between treatments. Ewes removed as shy feeders and joined on pasture recorded a 33% lower pregnancy rate but 17% higher rate of multiple fetuses than pen-joined ewes. **Conclusions.** Ewes can be fed complete high-grain maintenance rations at 2-day intervals during joining without a reduction in reproductive traits or wool quality compared with daily feeding. **Implications.** Producers may save labour with longer feeding intervals; however, greater monitoring and management intervention may be needed to maintain ewe liveweight when fed long term. Impacts in flocks without acidosis, on ram fertility when feeding is prolonged pre-joining, and with different diets require study.

Keywords: drought, embryo mortality, feedlots, fertility, nutrition, reproduction, sheep, wool.

Introduction

Nutrition during the periconceptional period is a key influence on the reproductive performance of ewes. The potential for manipulation of nutrition to improve reproduction is particularly relevant during drought or in housed situations where ewes are completely hand fed. During drought, the general aim is to feed ewes to maintain maternal liveweight, rather than achieve growth. The current recommendations for complete-ration feeding during drought are to feed every second or third day, rather than daily, to reduce labour costs and the incidence of shy feeders. It is also recommended to manage sheep in group-fed pens or yards (containment or drought-lot feeding), rather than large paddocks, to reduce energy expenditure from walking and to protect pastures from over-grazing (Bell *et al.* 2016; DEDJTR 2018). The recommendation against daily feeding is based on evidence from wethers (Franklin and Sutton 1952) and unmated ewes (Briggs *et al.* 1957) showing higher rates of mortality due to more variation in intake between sheep when fed daily rather than weekly. However, the impact of feeding frequency on the reproductive rate of ewes has received limited study.

Complete ration feeding at longer than daily intervals (>24 h) results in cycles of above-maintenance intake then fasting. During the periconceptional period, both above-maintenance intake (Cumming *et al.* 1975; Parr *et al.* 1987; Robertson *et al.* 2015*b*) and under-nutrition (Abecia *et al.* 2015) have been shown to increase the rate of embryo mortality in ewes, potentially reducing pregnancy rates. Parr *et al.* (1987) reported a 20% reduction in pregnancy rate due to daily feeding at twice maintenance compared with maintenance. However, twice-maintenance feeding has not consistently reduced pregnancy rates (Muñoz *et al.* 2008), and 3 days of fasting has produced a variable effect (Blockey *et al.* 1974). One report indicated no reduction in either the number of ewes lambing or lambing percentages by feeding ewes three times per week during pregnancy compared with daily (Jordan and Hanke 1963). We found only one report that evaluated frequency of feeding before and during joining; that study provided a base ration daily, with a concentrate supplement fed daily or on alternate days, with no adverse impact on reproduction of less frequent supplementation (Ben Khlil *et al.* 2017). However, the numbers of ewes in those studies were inadequate to assess pregnancy outcomes accurately, and the impact of complete ration feeding at longer than daily intervals during joining was not evaluated.

Poor reproductive performance has been associated with containment feeding despite the general aim of feeding to maintenance requirement, particularly during dry seasons, to minimise costs. Anecdotally, pregnancy rates from <50% to >90% have been reported in Australia, with the causes of poor results often unknown. Containment-fed ewes have produced 6–21% fewer lambs per ewe than ewes supplemented in paddocks (Ashton and Hannay 1984; Morbey and Ashton 1990); however, it is unclear whether this was due to different nutritional levels or to a factor related to containment feeding or husbandry. Given the known adverse impacts of high and low feed intake on rates of conception and embryo mortality, evaluation of feeding strategy during the periconceptional period is warranted to ensure that the recommendations provided to industry promote reproductive efficiency. Therefore, the aim of the present study was to quantify the impact of feeding once daily versus every second day (i.e. 2 days' ration fed on alternate days) at maintenance levels on the reproductive performance and overall productivity of ewes when joined under commercial containment conditions.

Materials and methods

Experimental design

The study was performed during 2021 on a commercial property 40 km north of Wagga Wagga, southern NSW, Australia, with approval from the Charles Sturt University Animal Care and Ethics Committee (approval no. A20301). Ewes were pen-fed during a March–April autumn joining (mating), within the natural breeding season, subsequently grazing pastures until the end of lambing. Two treatments were imposed during joining: daily feeding or feeding every second day, at maintenance levels of energy. A randomised block design was used to provide four replicates of each treatment, with the group-fed pen the experimental unit.

Sheep management

A flock of 800 mature Merino ewes was used (2.5, 3.5 and 4.5 years old, with an age distribution of 35%, 26% and 39%, respectively). The ewes had been shorn in early February 2021, when they were drenched with Hat-Trick® (Boehringer Ingelheim, Sydney, NSW, Australia) for broad-spectrum worm control and vaccinated with Glanvac 6 (Zoetis Australia, Sydney, NSW, Australia) against *Corynebacterium pseudotuberculosis* and clostridial diseases. Sexually experienced Merino rams (n = 17) aged 2.5–4.5 years were similarly drenched and vaccinated on 31 January 2021. The rams had been shorn in late October.

A timeline of the pen-feeding period is shown in Table 1, with the number of ewes remaining in each treatment at each stage shown in Table 2. Ewes and rams were introduced to barley grain (*Hordeum vulgare* L.) over 14 days while grazing crop stubble. The ewes were then stratified on age before random allocation to pens (n = 100 ewes/pen), and the two pens within each replicate block were randomly allocated to either treatment group. The ewes were placed in group-feeding pens at Day −17, where Day 0 was the day that rams were introduced to ewes. The different feeding frequencies commenced on Day −14, such that any effects of frequency in the oestrous cycle prior to mating were captured.

After excluding three rams for health reasons, rams (n = 14) were fitted with crayon harnesses (Mating Mark; Rurtec, Hamilton, New Zealand) then randomly allocated to replicate within treatment and placed in pens with the ewes (two per pen for each treatment in Replicates 1, 2 and 4; one per pen in Replicate 3) early on Day 0 (12 March 2021). Crayon marks were used to indicate which ewes were mounted and therefore assumed to be mated during Days 0–3, and blood samples were collected from these ewes on Days 3, 6 and 12. The crayon colour was changed on Day 14, and raddle marks were recorded on Days 14, 19, 24 and 31. All ewes were removed from the pens to grazing on Day 31, which was 14 days after the first 17 days of mating. This

Table 1. Timeline of the experiment indicating key activity and measurements, where Day 0 is the introduction of rams to ewes.

9–22 Feb. Days −31 to −17	23 Feb. Day −17	12 Mar. Day 0		12 Apr. Day 31	29 Apr. Day 48	17 June Day 97	7 July Day 117	9 Aug. Day 150	13–18 Sept.
Key activity									
Introduce grain	Ewes into pens	Rams in with ewes		Ewes out of pens to graze	Rams removed	Pregnancy scan	Wool sampling	Lambing starts	Marking of lambs
Measurements									
	Weigh, CS	Weigh, CS (Day −3)		Weigh, CS		CS (Day 101)		CS (Day 139)	CS
		Crayon 1 (Day 3)	Crayon 2 (Days 14, 19, 24)	Crayon 3 (Days 31, 38)	Crayon				
		Rumen pH (Day −2), Blood (Days 3, 6, 12)							
Semen (Day −18)				Semen (Day 27)		Semen (Day 82)			

Where days of sampling/recording differ from days of key activities, they are presented in parentheses under the closest key date.
CS, condition scoring; Crayon, recording raddle marks, where crayons 1–3 are raddle colour changes; Rumen pH, sampling rumen fluid; Blood, collecting blood samples; Semen, collecting semen.

Table 2. Number of ewes in each treatment for analysis at each day of the experiment, where Day 0 is the introduction of rams to ewes.

Day of experiment	Activity	Feeding treatment		Total
		Daily	Alternate days	
Day −17	Allocation of ewes to pens	400	400	800
Day 0	Rams joined to ewes	7	7	14
Days −17 to 97	Ewes removed or excluded as dead, in poor health, missing or pregnant prior to study	11	8	19
Days −17 to 31	Shy feeders removed	42	35 (+1)[A]	77 (+1)
Day 31	End of pen feeding	347	356	703
Day 97	Pen-fed ewes removed if not pregnant	37	37	74
Day 97	Shy feeders removed if not pregnant	21	13	34
Day 117	Pen-fed ewes removed if conception occurred after Day 19[B]	61	63	124
Day 139	Pen-fed ewes pregnant by Day 19 placed in lambing plots	249	256	505
Day 139	Shy feeders placed in lambing plots	22	21	43
Marking	Pen-fed ewes condition scored	242	250	492

[A]Analysis excluded one ewe that escaped from pen into the shy feeder group.
[B]Conceived later or not raddled but pregnant, so date of mating was unknown.

allowed assessment of reproduction for ewes mated on the first oestrous cycle. The crayon colour was again changed on Day 31, and four harnessed rams remained with the single mob of grazing ewes to allow further returns to service to be detected. Crayon marks were recorded on Days 38 and 48, at which time the remaining rams were removed. One of these rams was euthanised on Day 48, because it was cast and found to have a penile/prepuce infection (balanoposthitis). Unfortunately, it was in Replicate 3, where both treatments had used only a single ram per pen during joining.

After release from pens, the ewes grazed as one flock on wheat (*Triticum aestivum* L.) stubble (3.3 ± 0.25 t dry matter (DM)/ha) for 2 days, with increasing access to mixed green pasture. This was done to avoid a sudden change in diet. Thereafter, the ewes grazed mixed green pasture (initially 708 ± 354 kg DM/ha).

Transabdominal ultrasonography was used to determine fetal number on Day 97 after ram introduction. Non-pregnant ewes and ewes not pregnant in the first 19 days of mating, based on raddle marks, were removed from further study. This allowed assessment of lamb performance when the conceptus was exposed to different feeding frequencies to at least the twelfth day of pregnancy. The remaining ewes ($n = 505$) continued to graze as one flock until Day 139 (29 July 2021), when they were drafted into their pen groups (n = 50–74 ewes) and placed into 5.3-ha paddocks for lambing. Treatment groups of ewes within each replicate were placed into adjacent paddocks, which had been subdivided from a larger paddock. The quantity of pasture on offer in each paddock was estimated before and at the end of lambing, from 30 visual estimates per plot, calibrated using 10 quadrat cuts (each 0.1 m^2) per paddock, which were dried at 60°C, then weighed (Haydock and Shaw 1975).

During the lambing period, ewes were checked daily, and assistance was provided to any ewes with clear signs of dystocia. Lambs were not tagged at birth owing to resource constraints, meaning neither dam nor birth type (single/twin) of individual lambs was recorded. Two weeks after the end of lambing, the sheep in each paddock were yarded separately, ewes were condition scored, and lambs were ear tagged and weighed, and their sex was recorded. The percentage lamb survival per paddock was calculated as the number of lambs present/number of fetuses placed in the paddock × 100.

During the pen-feeding period, ewes (n = 77) that were visually assessed as shy feeders (abdomen hollow at the flanks, indicating low gutfill) were removed from the pens and returned to pasture on Day −10 and Day 19. These ewes were grazed on lucerne (*Medicago sativa* L.) pasture during joining and mated as one group to non-experimental rams from Day 0. Fetal numbers were recorded as for the pen-joined ewes, but pregnant shy feeders grazed as a single separate group until lambing, when they were drafted into the two treatment groups and placed in small paddocks so that lamb production could be recorded.

Feeding and pen design

Each pen measured 30 m by 10 m, and each contained a double-sided feed trough of 12.2 m length, a self-filling water trough, and a 10 m by 5.4 m strip of 70% and 50% shade cloth. Due to COVID delays it was not possible to purchase a 2nd strip of 70% shadecloth, so 50% was used. The ewes, and rams separately, were introduced to barley grain in a paddock over a 14-day period with access to crop stubble. Ground limestone and coarse salt were added to the grain at a rate of 1% each. Once ewes were penned, the daily grain ration was increased to 700 g/ewe plus 10% wheat straw, with alternate-day feeding of ewes and rams commencing on Day −14. Sheep were fed from 08:00 in a single feeding session. Bales of straw were broken and spread on the ground to allow access by all sheep. Rainfall during Days 0–12 caused ewes in all pens to refuse straw, and signs of acidosis became prevalent. The proportion of ewes not coming to the trough for grain when fed was recorded. On the morning of release from pens (Day 31), ~400 g straw/ewe was fed, with no grain, to avoid a sudden change of feed and gorging on pasture.

A sample of barley grain was tested prior to commencement of feeding in order to estimate the quantity required. Samples of barley and straw were also collected weekly throughout the pen-feeding period, and then a bulked sample of each was analysed for nutrient and mineral composition. Mineral composition was analysed by the NSW Department of Primary Industries (DPI) Environmental Laboratory, Wollongbar, using microwave digest and ICP-AES or ICP-MS analysis (in-house methods P051, M670 and M671). Proximate analyses (% DM) were determined via near infra-red reflectance spectroscopy with a multi-purpose analyser (MPA; Bruker Optik, Ettlingen, Germany) and OPUS software (ver. 5.1) using calibrations developed by the NSW DPI Feed Quality Service, Wagga Wagga (AFIA 2007). Crude protein (CP) was estimated from nitrogen (N) as: CP (%DM) = N (%DM) × 6.25. Metabolisable energy (ME) was estimated from digestible organic matter in the DM (DOMD) as: lucerne ME (MJ/kg DM) = 0.203 DOMD (%) – 3.001 (AFIA 2007).

Weather data for the experimental period were sourced from station 072150 at Wagga Wagga, 38 km south of the experimental site (www.bom.gov.au; accessed 11 October 2021). Ambient temperature was also recorded onsite every 10 min from Day −3 to Day 30 in an unshaded area of an empty pen, using a data logger incorporating a Stevensen screen, attached to a post at 90 cm height (Tinytag data logger; Gemini Data Loggers, Chichester, UK).

Sheep measurements

All ewes were weighed (unfasted) and their condition scores recorded on a 1–5 scale (1 = emaciated, 5 = obese) (Jefferies 1961) at allocation to pens on Day −17. Thirty randomly selected ewes were subsampled per pen and reweighed and their condition scores recorded on Day −3. These same ewes were measured again on Day 19 after ram introduction. All ewes remaining in the pens were weighed and scored on Day 31 when removed from pens, and were scored at Day 101 following pregnancy scanning. The condition score of ewes was then recorded pre-lambing (Day 139) and post-lambing for ewes still present.

The presence of clinical ruminal acidosis was recorded daily during pen-feeding, and assessment of fresh faeces (Dickson and Jolly 2011) in each pen was made from Day −7. Visual estimation was made of the percentage of faeces in each of the following scores: 1 (hard pellets), 2 (thick faeces with pellet formation), 3 (soft, lacking pellets), 4 (runny faeces) to 5 (liquid faeces).

Blood samples were collected from all ewes mated between introduction of rams (Day 0) and sampling on Day 3, as indicated by crayon marks. The same ewes were sampled on Days 3, 6 and 12 by jugular venepuncture using 9-mL lithium heparinised BD Vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Sampling commenced at 11:00 on each occasion, 2 h after the completion of feeding. Blood samples were stored on ice before centrifugation, with plasma separated and frozen at −20°C until analysis. Progesterone was measured in duplicate by using a commercial radioimmunoassay kit (IM1188, Progesterone RIA Kit; Beckman Coulter Australia, Sydney, NSW, Australia). The samples were assayed in duplicate 50-μL aliquots and run in three assays. The limit of detection was 0.20 ng/mL. Intra-assay coefficients of variation (CVs) for samples containing 1.33 and 5.51 ng/mL were 7.6% and 4.6%, and inter-assay CVs were 9.4% and 4.8%, respectively.

The rams (n = 17) were randomly allocated to treatment and fed in two separate treatment pens from Day −14. A semen sample was collected from each ram by electro-ejaculation on Day −18 (prior to treatments commencing) and assessed for morphology, and rams were physically examined to minimise the risk of using subfertile rams. One ram was excluded from joining because of poor sperm morphology, and two rams were excluded with mild ruminal acidosis at Day −12, leaving 14 rams available for joining. Semen samples were again collected on Day 27 to assess potential treatment effects, and on Day 82 (i.e. 52 days after removal from pens) to assess recovery after being fed at the two different frequencies. One ram with blood-stained semen (haemosemen) at all collections remained with ewes until removal from pens, because at the time, the cause and impact on ram fertility were unknown. This ram was subsequently diagnosed with *Histophilus somni* infection of the vesicular gland and, when mated with ewes 7 months later, found to have low fertility.

For assessment of wool quality, a wool sample was clipped from the midside of 10 pregnant ewes from each pen on Day 117. Wool samples were sent to a commercial laboratory (Riverina Wool Testers, Wagga Wagga) for estimation of yield, fibre diameter, staple strength and staple length, using International Wool Textile Organisation methods (IWTO 2015).

Statistical analyses

Data for 19 ewes that died pre-joining or were removed from the experiment for health reasons (including pregnant pre-experiment, abnormal udder) were excluded, leaving 781 ewes for analysis. Analyses of ram data excluded Replicate 3 because the ram euthanised was the sole ram in one pen; and for semen data only, one alternate-day treatment ram with haemosemen associated with infection, and one daily-treatment ram with a large decline in semen quality.

The experimental unit for analyses was the pen group. Data were assessed for assumptions of normal distribution and homogeneity following analysis using Genstat 21th edition (VSN International, Hemel Hempstead, UK). Lamb survival was not normally distributed and so was analysed using a Wilcoxon rank-sum test, with median values presented. Pasture data at lambing were transformed by natural logarithm to equalise variances, and analysed using linear mixed modelling with time × treatment as the fixed effects. Progesterone concentrations were similarly transformed and analysed, using the fixed effects of year born + return to service × date × treatment, and the random terms replicate + ewe. The term replicate accounts for pen as the experimental unit, with individual ewe included to consider repeated measurements on the individual. Back-transformed data are presented as geometric mean (geometric mean = $e^{\text{mean ln}}$), and a least significant ratio (l.s.r. = $e^{(\text{l.s.d. ln})}$) was used to compare ratios between individual means.

Linear mixed modelling was also used for: wool quality variables, which were analysed using year born × treatment; ram variables, which used date × treatment, with pre-treatment value included as a covariate for the analysis of percentage normal semen; lamb marking weight, which used sex × treatment; ewe weight and condition score at allocation and their change until removal from pens, which used year born × treatment (pen-fed or shy feeder status was included as a fixed effect only at allocation); condition score over time for ewes present at lamb marking, which used year born + fetal code × time × treatment. All models used replicate as the random term, with the addition of either ewe or ram identity where relevant to account for repeated measures on individuals. A *post hoc* Bonferroni correction was applied to determine differences between individual means. A P-value of 0.05 was considered significant. Results are presented as mean ± standard error.

Data for proportions (shy feeders, day mated, returns to service, pregnancy, and multiple fetuses) were analysed using generalised linear mixed modelling (GLMM) with year born + treatment as the fixed effect and replicate as the random effect. The effect of ram percentage of 1 or 2 per ewe joined on mating progress, returns to service and pregnancy rates also used GLMM, but used ram percentage as the fixed term and pen as the random term due to fewer pens using one ram than two. The logit transformation meant that standard errors for back-transformed means were not available. Descriptive comparisons were used to compare shy feeder groups with the pen-joined groups, with means and frequencies reported.

Results

Weather

Weather was mild during the joining period, with only 1 or 2 days having temperatures >32°C during February and March. The on-site mean maximum daily temperature during the pen-fed joining period (Days −3 to 31) was 24.5°C (±4.6°C) and the minimum 11.5°C (±3.9°C); however, sheep were observed to prefer the shaded area of pens during the day. Substantial rainfall of 10–32 mm occurred on each of 4 days during the joining period, all between Days 0 and 12. Weather during August, when lambing, was not considered of high chill index (Donnelly *et al.* 1997), with a mean daily minimum of 3.3°C (±1.3°C), maximum 15.7°C (±0.5°C), 3 pm wind speed of 17.8 ± 1.3 km/h, and only 2 days of rainfall >5 mm.

Table 3. Nutritive and mineral composition of the barley grain and wheat straw fed to ewes.

	Barley grain	Wheat straw	Limit of reporting
Dry matter (DM, %)	93.1	91.6	0.5
DM digestibility (%)	86	40	39
Digestible organic matter in DM (%)	84	40	38
Neutral detergent fibre (%)	19.0	79	10
Acid detergent fibre (%)	5.0	52	4
Crude protein (%)	10.5	3.1	2
Metabolisable energy (MJ/kg DM)	13.1	5.2	4.3
Aluminium (mg/kg)	6.5	120	5.0
Arsenic (mg/kg)	<5	<5	5.0
Boron (mg/kg)	<4	<4	4.0
Calcium (%)	0.046	0.17	0.001
Cadmium (mg/kg)	<0.2	<0.2	0.2
Cobalt (mg/kg)	<0.05	0.074	0.05
Chromium (mg/kg)	<0.2	1.4	0.2
Copper (mg/kg)	3.6	2.0	0.2
Iron (mg/kg)	42	110	0.6
Potassium (%)	0.71	0.79	0.0004
Magnesium (%)	0.13	0.053	0.001
Manganese (mg/kg)	17	110	0.01
Molybdenum (mg/kg)	0.36	0.39	0.05
Sodium (%)	0.0060	0.0052	0.0005
Nickel (mg/kg)	<0.7	<0.7	0.7
Phosphorus (%)	0.41	0.058	0.001
Lead (mg/kg)	<2	<2	2
Sulfur (%)	0.12	0.075	0.006
Selenium (mg/kg)	0.06	<0.05	0.05
Zinc (mg/kg)	20	3.4	0.8

Feed quality and refusals

The nutritive value of the barley grain and wheat straw is shown in Table 3. Both the grain and straw were largely consumed within 1 h of feeding, such that there were no or low refusals except on Days 0 and 2 and 9–12, when up to ~50% of straw was refused due to rain.

Health

Clinical acidosis was present in all pens after Day 0, and soft faeces (score >3) persisted for 3 weeks at a low incidence (estimated 1–5% of fresh faeces in pens). The rain and acidosis during Days 10–21 were associated with 5–24% of ewes in most pens ceasing to approach the trough when fed, although in one pen (Pen 8, daily treatment) the average incidence was 40% (varying between 20% and 67% per day) of ewes. Feeding behaviour in all pens had largely returned to normal by Day 22. During the feeding period, four of the 800 ewes (three from daily feeding and one from alternate-day feeding) died or were euthanised due to acidosis. One ram in the alternate-day treatment was euthanised on Day 48 after detection of balanoposthitis, which would have prevented mating.

Some of the ewes ($n = 77/781$) were removed from pens as shy feeders. The proportion removed was similar between treatments (0.07; $P = 0.486$) but differed among age groups ($P < 0.001$). It was negligible in 4.5-year-old ewes (0.01), but was higher in 2.5- and 3.5-year-old ewes (0.12 and 0.19, respectively).

Ewe weight and condition score

The geometric mean liveweight and least-square mean condition score of ewes at allocation (Day −17) were 54 kg and 3.0 ± 0.04 and were similar between feeding frequency treatments. Ewes aged 3.5 years were in poorer ($P < 0.05$) condition (2.7 ± 0.04; 51 kg) than younger (3.1 ± 0.04; 53 kg) or older (3.2 ± 0.04; 58 kg) ewes. Ewes that were later removed as shy feeders were 6 kg lighter and in poorer condition at allocation (2.8 ± 0.05) than those remaining in pens (3.2 ± 0.03).

For those ewes not removed as shy feeders, daily feeding resulted in minimal loss of liveweight (−1.3 ± 0.3 kg) between allocation and removal from pens, but the loss was greater ($P = 0.003$) than alternate-day feeding (−0.3 ± 0.3 kg). Weight loss over the feeding period was not different ($P = 0.509$) between ewes that returned to service after mating by Day 19 (−0.7 ± 0.3 kg) and those that did not return (−0.8 ± 0.4 kg). Mean changes in condition score for both treatments were negligible (<0.2 score).

For pen-fed ewes remaining at lamb marking, condition score was similar ($P > 0.05$) for both feeding frequencies at all time points (allocation, removal from pens, pregnancy scanning, pre-lambing and lamb marking). There was no interaction of fetal number, time and treatment, but multiple-bearing ewes were in better ($P < 0.05$) condition at removal from pens (by 0.21 score), and poorer condition score at lamb marking (−0.24 score), than single-bearing ewes. The raw mean condition score of ewes removed as shy feeders was 3.2 ± 0.03 at pregnancy scanning, and for those pregnant, 3.2 ± 0.07 pre-lambing.

Reproductive performance

Of the 703 ewes not removed as shy feeders and remaining in the study, raddle marks indicated 92% mated by Day 19 and 96% mated by removal from pens (Day 31), with 97.6% mating by Day 48. Only 2.4% of ewes failed to be raddled, although most of these (12/17) were subsequently scanned as pregnant, meaning that at least 99.3% of ewes mated with, or were mounted by, rams. Feeding frequency did not alter the proportion of ewes mating by Day 19, although age of ewe did ($P < 0.001$), with 10% fewer 2.5-year-old ewes mating than older ewes. This influence was still present at Day 30 but not at Day 38 of joining.

The proportion of ewes returning to service after first mating by Day 19 of joining was similar between feeding frequencies (Table 4) and among ewe ages. Considerable variation occurred among pens, with Pen 2 (0.31; daily feeding, replicate 1) and Pen 6 (0.41; alternate-day feeding, replicate 3) producing higher rates of return than other pens (0.16–0.24). Pen 2 was associated with lameness in one of the two rams present, and ewes in Pen 6 were mated to a single ram found to have balanoposthitis at the end of joining. Returns to service were not elevated in Pen 1 (alternate-day feeding, replicate 1) where one of two rams present produced haemosemen at all collections.

Pregnancy rate and proportion of ewes with multiple fetuses were similar ($P > 0.05$) between feeding frequency treatments for ewes joined in the pens (Table 4). Age of ewe did not alter the proportion pregnant, but a larger ($P = 0.470$) proportion of ewes aged 4.5 years bore multiples than younger ewes (0.53 vs 0.37 of those pregnant). The survival, weight and proportion of female lambs at marking were similar ($P > 0.05$) between feeding frequencies. Ample live pasture was on offer at lambing, and was similar between treatments, but the geometric mean for herbage available was lower ($P < 0.001$) pre-lambing than post-lambing (1895 vs 2852 kg DM/ha).

The proportion pregnant of ewes removed as shy feeders (0.53; 43/77) was 33% lower than that of ewes remaining in pens. However, for those ewes scanned as pregnant, the proportion of ewes with multiple fetuses was 17% higher for shy feeders (0.60; 26/43) than for those remaining in pens.

Progesterone concentrations

The mean progesterone concentration was 18% higher ($P = 0.015$) in ewes fed daily than on alternate days; it increased ($P < 0.001$) between successive sampling dates (geometric means Day 3, 6 and 12: 0.94, 1.93 and 5.61 ng/mL, respectively); it was higher ($P < 0.001$) in 4.5-year-old than younger ewes; but it was not reduced ($P = 0.769$) in ewes that returned to service. There were no significant interactions. Progesterone concentrations were <2 ng/mL in 55 of 111 ewes at Day 6, and <2 ng/mL in 2 of 111 ewes at Day 12.

Ram performance

The condition score of rams was similar between feeding frequencies but increased from late joining to post-joining (Table 5). Mean scrotal circumference and percentage of semen with normal morphology were also similar between treatments but declined over time, with no interaction ($P > 0.05$) between time and treatment.

Pens with one ram (Replicate 3) rather than two rams had a lower ($P < 0.002$) proportion of ewes mated by Day 19 (0.86 vs 0.94), but by Day 30 the proportions were similar ($P = 0.241$) (0.94 vs 0.97). The proportion of ewes returning to service after mating by Day 19, pregnant or bearing multiples, was not different at the lower ram percentage.

Table 4. Mean reproductive performance of ewes joined in containment and fed daily or every second day to Day 30 of joining, and lambing performance of ewes pregnant by Day 19 of joining.

Variable	Daily feeding	Alternate-day feeding	*P*-value
No. of ewes joined in pens[A]	347	356	
Proportion returning to service from 1st mating when mated by Day 19	0.24 (74/315)	0.23 (76/332)	0.827
Proportion returning to service twice after mating by Day 14	0.05 (15/257)	0.04 (12/261)	0.559
Proportion pregnant of total ewes	0.89 (310/347)	0.89 (319/356)	0.912
Proportion with multiple fetuses of total ewes	0.36 (130/347)	0.39 (143/356)	0.467
Proportion with multiple fetuses of pregnant ewes	0.41 (130/310)	0.44 (143/319)	0.487
No. of ewes to lambing plots[B]	249	256	
Proportion of ewes with multiple fetuses to lambing plots	0.42 (101/249)	0.45 (109/256)	0.921
No. of lambs marked	248	243	
Lamb weight at marking (kg)	11.9 ± 0.4	12.1 ± 0.4	0.442
Median lamb survival (%)	70.9	66.2	0.343
No. of lambs marked per ewe lambing	1.00 ± 0.04	0.95 ± 0.04	0.378
Proportion of female lambs marked	0.52	0.57	0.265

[A]Excludes shy feeders, which were removed from pens, and ewes that were removed or excluded for health reasons or were pregnant prior to the experiment.
[B]Only ewes pregnant up to Day 19 of joining were placed in lambing plots, ensuring that the conceptus had been exposed to the feeding treatments to at least the twelfth day of pregnancy.

Table 5. Mean (±s.e.) condition score, scrotal circumference and normal semen percentage pre-treatment (Day −18), late joining (Day 27) and post-joining (Day 82) for rams fed daily or every second day.

	Treatment		Time				
	Daily	Second day	Pre-treatment	Late joining	Post-joining	*P*-value treatment	*P*-value time
Condition score	3.0 ± 0.1	3.2 ± 0.1	–	2.9 ± 0.0a	3.2 ± 0.1b	0.166	0.047
Scrotal circumference (cm)	35.2 ± 0.6	34.2 ± 0.6	37.5 ± 0.6b	33.5 ± 0.6a	33.1 ± 0.6a	0.162	<0.001
Normal semen (%)[A]	72 ± 5	79 ± 5	–	80 ± 4b	71 ± 4a	0.305	0.025

[A]The pre-treatment sample was used as a covariate; excludes one control ram whose normal semen declined from 90% to 45% then 11%.
Within rows within factors, means followed by the same letter or no letter are not significantly different at $P = 0.05$.

Wool quality

Mean fibre diameter (17.9 ± 0.3 μm), staple length (44 ± 1 mm), staple strength (45 ± 2 N/ktex) and yield (72% ± 1%) were similar between treatments. Wool from 5-year-old ewes was ~1 μm broader and 8 N/ktex stronger ($P < 0.05$) than from 3- or 4-year-old ewes.

Discussion

To our knowledge, this is the first large-scale study to investigate the impact of frequency of complete ration feeding during joining on the productivity and reproductive performance of ewes to lambing. Maintenance feeding of ewes every second day, rather than daily, did not reduce lamb production, ewe health or wool quality, so is a suitable strategy to reduce labour costs when feeding during joining.

Embryo mortality did not appear to be increased by alternate-day feeding, as indicated by the rates of return to service, pregnancy and fecundity. Feeding every second day results in alternate days of twice-maintenance intake then fasting. Twice-maintenance levels of intake when fed daily can cause embryo mortality through reduced circulating progesterone concentrations, with embryos most sensitive 11 and 12 days after mating (Parr 1992). In that experiment, the average feeding level was twice the level used in our experiment.

However, feeding a maintenance ration in one event rather than in several events during a day has also been shown to reduce progesterone concentrations (Vasconcelos *et al.* 2003). In the present study, progesterone concentrations were reduced at Day 12 of joining by alternate-day feeding, but not below the 2 ng/mL shown as the requirement for maintenance of embryos (Parr *et al.* 1987). This may explain the lack of effect on pregnancy rates. High progesterone concentrations may also have occurred due to the high condition score of ewes (Viñoles *et al.* 1999). It remains unclear whether the reproduction of ewes in poorer body condition with few twin ovulations, as may occur during drought, would be impacted by infrequent feeding.

Occurrence of ruminal acidosis, resulting in a proportion of ewes being reluctant to eat during the peak periconceptual period and possibly changing meal size, may have contributed to alternate-day feeding frequency not reducing progesterone concentrations to levels that may have caused embryo loss. It also seems that any endotoxaemic effects of acidosis inducing an inflammatory response including prostaglandin (PGF2a) release (Smith *et al.* 2019) and lysis of the corpora lutea, reducing conception rates, were not a notable factor in this study. However, the high rates of pregnancy and fecundity recorded in the study do suggest that relatively minor degrees of acidosis (not requiring treatment) in ewes during the periconceptual period do not necessarily prevent high pregnancy rates.

The fecundity (proportion of pregnant ewes bearing multiple fetuses) of ewes was not increased by alternate-day feeding. Banchero *et al.* (2021) suggest that suboptimal protein prevents cereal grains from producing a sufficient glucose entry rate to cause an increase in ovulation rate. It remains unclear whether a reduced feeding frequency would alter fecundity for ewes fed non-cereal diets. However, cereal-based rations were the most commonly used feedstuff in Australian drought-feeding situations reported in a producer survey (Robertson *et al.* 2021).

The survival of lambs from ewes pregnant by Day 19 of joining was similar between ewes fed daily and every second day. An increase in lamb survival associated with feeding at 60% of maintenance from mating to 39 days post-mating has been reported (Muñoz *et al.* 2008). Those authors suggested that the increase may have been associated with greater physiological maturity of lambs at birth due to increased length of gestation, or an increased ability to absorb colostral antibodies. However, there is no indication in the present study that short-term fasting associated with a two-day feeding interval when fed at maintenance improves lamb survival.

The performance of rams was not reduced by less frequent feeding. The decline in scrotal circumference throughout the joining period was probably due to a reduction in intake and consequent reduction in sperm production, which may be expected in working rams (Martin *et al.* 2011), combined with a reduction in semen stored due to frequent ejaculation. Sperm abnormalities may result from vitamin A deficiency associated with prolonged feeding of cereal grain (Abdulkareem *et al.* 2005). However, the rams had access to a small quantity of green forage prior to pen-feeding, so vitamin A deficiency seems improbable. Despite the apparent decline in morphology, ram libido and fertility appeared to be adequate because almost all ewes were mated, and the conception rate was similar to reports for commercial flocks (Kleemann and Walker 2005; Allworth *et al.* 2017). The conception rate, comprising the net effect of fertilisation and embryo mortality, obtained from the semen of individual rams is known to vary widely (Santolaria *et al.* 2014). Joinings to single rams therefore entail some risk, as observed with one ram developing balanoposthitis; however, adequate replication prevented confounding of experimental outcomes in the present study.

A reduced frequency of feeding is known to cause increased variation in intake between sheep and, so, greater loss of liveweight among a proportion of the flock (Franklin and Sutton 1952; Briggs *et al.* 1957). In the present study, ewe weight was largely maintained, due to the removal of shy feeders to maintain ewe welfare. A longer period of feeding may have caused a greater reduction in the liveweight of daily fed ewes, indicating a need for continuing monitoring and adjustment of feeding level.

Daily feeding did not increase the incidence of shy feeders, perhaps because the length of the feed trough (24 cm per ewe) allowed access by all ewes, and was above the minimum 5 cm per 45 kg wether at which weight change stabilises when maintenance feeding (Dundon and Mayer 2015). The removal of 7% of ewes as shy feeders was consistent with an industry perception of 5% (Robertson 2020). The cause of the difference in the rate of shy feeders among ewes of different ages is unclear, but may have been different feeding histories, liveweight or social interactions (Rice *et al.* 2016). Most shy feeders were detected within 1 week of pen-feeding, but removal of some after 5 weeks indicates either that a more efficient means of identifying shy feeders is needed, or that these developed after removal of the earlier group, perhaps due to changing social status within pens, or because of feed aversion due to acidosis.

Minimising the proportion of shy feeders is necessary to maximise reproductive efficiency. The low pregnancy rate of the shy feeders removed from pens may have been due to weight loss and/or low condition score. It may also have been due to sub-fertility in the non-experimental rams used, or embryo loss associated with recent weight loss, considering that mortality occurring at 20–30 days of gestation reduced subsequent pregnancy rates by 57% in one study (Sawyer and Knight 1975). The increased fecundity of the shy feeder ewes was probably due to them being joined on live lucerne pasture (Robertson *et al.* 2015*a*) with a higher nutritional level than pen-fed ewes. The higher fecundity of the shy feeders than containment-joined ewes indicates caution against unnecessary use of containment where pasture conditions may provide a nutritionally superior alternative, consistent with early studies (McManus *et al.* 1976).

Wool production was not altered by feeding every second day. Feeding frequency has previously been shown not to alter fleece weights (Briggs *et al.* 1957) or fibre diameter (Hodge *et al.* 1981). By contrast, feeding cereal grain once weekly, rather than daily, has been reported to increase wool growth by 24% for the period fed, probably associated with high intake causing a larger portion of protein to escape the rumen to be digested in the small intestine (Langlands 1973). The response varies with feed type (Masters *et al.* 2002). In the present study, any increase in wool growth rate was probably masked by the short period in which ewes were differentially fed.

Conclusions

Feeding a diet high in cereal grain at maintenance levels daily or every second day from 14 days before joining to Day 30 of a natural joining period did not change reproductive performance or lamb and wool production in ewes. Although high rates of reproduction were achieved, acidosis was a considerable health problem, and further evaluation of feeding frequency is needed to replicate the findings, including other diets commonly used by commercial breeders.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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