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Calcium and magnesium supplementation of ewes grazing pasture did not improve lamb survival

Susan M. Robertson[A,B,C,*], Shawn R. McGrath[A,B,C], Samuel Scarlett[A], Marie Bhanugopan[A,B], Janelle E. Hocking Edwards[D], Emma Winslow[D], Serina Hancock[E], Andrew N. Thompson[E], Gordon Refshauge[F] and Michael A. Friend[G]

For full list of author affiliations and declarations see end of paper

*Correspondence to:
Susan M. Robertson
School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Boorooma Street, Wagga Wagga, NSW 2678, Australia
Email: surobertson@csu.edu.au

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ABSTRACT

Context. Clinical deficiencies of calcium and magnesium may result in the metabolic disorders hypocalcaemia and hypomagnesaemia, resulting in ewe and lamb mortality. However, the contribution of subclinical deficiencies to perinatal lamb mortality in grazing flocks is unclear. **Aims.** To test the hypothesis that calcium and magnesium supplementation during the lambing period would increase lamb survival to marking age. **Methods.** In 2017, an on-farm study used five flocks across New South Wales, South Australia and Western Australia. On each farm, twin-bearing mature Merino ewes (n = 400–600) grazing pasture were allocated to two replicates of control and supplemented treatments. The supplemented groups were offered 30 g/ewe per day of a loose lick containing magnesium chloride ($MgCl_2(H_2O)_6$), calcium sulfate ($CaSO_4{\cdot}(H_2O)_2$, and salt (NaCl), in the ratio 12.5:32.5:55.0, designed to have a low dietary cation–anion difference (−390 meq/100 g). A second study was conducted in 2018 on one farm to test the form of supplement. This study used two replicates of three treatments: control; a low-dietary cation–anion difference supplement as used in 2017; and a standard lime, Causmag (calcined MgO) and salt loose mix (ratio 1:1:1). Mature twin-bearing composite ewes (n = 600) were allocated to groups and those supplemented were offered minerals for the last month of pregnancy and during the lambing period. Blood and urine samples were collected in both experiments for analyses of mineral concentrations. **Key results.** In the 2017 study, only two flocks consumed >10 g/ewe of supplement per day, and supplementation did not increase lamb survival to marking age in these flocks. In the 2018 study, the mean consumption of supplement was 18 or 20 g/ewe per day. Of non-supplemented ewes, 61% were deficient in plasma calcium (≤90 mg/L) and 17% were deficient in magnesium (≤18 mg/L) at Day 140 after the start of joining. Lamb survival was not increased by supplementation and was 77 ± 3.8% in both treatments. **Conclusions.** Calcium and magnesium supplementation did not increase lamb survival. **Implications.** Lamb survival was not increased by calcium and magnesium supplementation; however, evaluation under a wider range of grazing conditions with adequate supplement intake is required.

Keywords: dystocia, hypocalcaemia, hypomagnesaemia, minerals, mortality, nutrition, reproduction, survival.

Introduction

Perinatal lamb mortality remains a significant source of reproductive loss, and nutritional management is a key factor in minimising these losses (Hinch and Brien 2014; Dwyer *et al.* 2016). Clinical deficiencies of calcium (Ca, <68 mg/L; Caple *et al.* 1988) and magnesium (Mg, ≤12 mg/L; CSIRO 2007) may result in the clinical metabolic disorders of hypocalcaemia or hypomagnesaemia, which are likely to cause the death of ewes and their fetus(es) or dependent lambs. The incidence of these disorders within ewe flocks is typically low, less than 3%, and sporadic, although death rates during outbreaks may be

20% (Herd 1966; Larsen *et al.* 1986; Caple *et al.* 1988). In contrast, subclinical Ca and Mg deficiencies are common in grazing ewes, with one-third of flocks across southern Australia containing ≥20% of ewes with inadequate concentrations pre-lambing reported in one study (Hocking Edwards *et al.* 2018). Subclinical deficiency of Mg is defined as a blood Mg concentration of <18 mg/L (0.74 mmol/L), and not showing clinical signs (McCoy *et al.* 2001). Subclinical calcium deficiency is defined as blood Ca of <90 mg/L (2.0 mmol/L) and not showing clinical signs (Suttle 2010). However, it is unclear whether subclinical deficiencies elevate perinatal lamb mortality (Friend *et al.* 2020).

Potential mechanisms by which subclinical Ca and Mg deficiencies may increase perinatal lamb mortality include dystocia, increased duration of parturition and impaired ewe or lamb behaviours. Dystocia is one of the key causes of perinatal lamb mortality (Hinch and Brien 2014) and prolonged parturition reduces lamb survival, by 8.7% in one report (Darwish and Ashmawy 2011). Supplementation of clinically normal ewes with Ca and Mg in the form of lime and Causmag showed a trend to reducing the duration of parturition for second-born twins (Ataollahi *et al.* 2021), and this may have been associated with improved energy regulation (Ataollahi *et al.* 2018). Inducing hypocalcaemia in ewes (Robalo Silva and Noakes 1984) demonstrates a role for Ca in dystocia, since an induced calcium deficiency (49 mg/L) during early labour reduced uterine activity, although this did not occur during later stages.

Low dietary concentrations of both Ca and Mg may be associated with a reduction in temperature regulation in rats and humans (Goubern *et al.* 1993; Zemel 2004), and dystocia also results in impaired thermoregulation in lambs (Darwish and Ashmawy 2011). Poor temperature regulation may contribute to the development of hypothermia in lambs exposed to cold weather, increasing mortality rates. Supplementation of clinically normal ewes with Ca and Mg also improves immune function in their lambs (Ataollahi *et al.* 2018), which might be expected to contribute to higher lamb survival.

The evidence therefore suggests potential for subclinical plasma Ca and Mg concentrations to reduce lamb survival. Supplementation with Ca and Mg in either lime/Causmag or low-dietary cation–anion difference (DCAD) forms has been shown to improve the mineral status of late-pregnant twin-bearing ewes grazing cereal crops (Masters *et al.* 2019), but that study did not measure lamb survival. The low-DCAD form of supplementation is thought to increase Ca mobilisation from bones, or increase absorption from the intestines (see review by Friend *et al.* 2020). However, there are no studies in sheep evaluating the effectiveness of low-DCAD supplements to increase lamb survival, to our knowledge, and only one study has evaluated lime/Causmag supplementation for lamb survival, with no increase reported (McGrath *et al.* 2015). There is a need to further evaluate the use of Ca and Mg supplementation in clinically normal ewes to determine whether, and under which conditions, lamb survival may be increased. The hypothesis of the current studies was, therefore, that providing Ca and Mg supplements in the form of a low-dietary cation–anion difference (DCAD) supplement or as a standard supplement to clinically normal pregnant ewes grazing pasture would increase lamb survival.

Materials and methods

The experimental procedures were approved by the Charles Sturt University Animal Care and Ethics Committee, with Approval numbers A17030 and A18013.

Experiment 1

The design was a randomised block with two replicates of two treatments, repeated on five experimental sites (properties) at Holbrook and Tullamore (NSW), Robe and Kingston (SA) and at Pingelly (WA). A non-supplemented control was compared with a loose-lick mineral-supplementation treatment offered to lambing ewes. The mineral supplement was formulated to provide a low-DCAD (−390 meq/100 g), and comprised magnesium chloride ($MgCl_2(H_2O)_6$), calcium sulfate ($CaSO_4{\cdot}(H_2O)_2$, and salt (NaCl), in the ratio of 12.5:32.5:55.0, fed at a rate of 30 g/ewe per day, with a target of 100% consumption. An intake of 20 g of supplement was estimated to provide 25% of Mg, 40% of Ca, and 487% of sodium (Na) requirements. The supplement was manufactured by DSM Nutritional Products, Australia (www.dsm.com).

Sheep management, supplementation and measurements

Commercial Merino flocks where ewes had been scanned using transabdominal ultrasound to determine fetal number were used as experimental sites. At each site, between 400 and 600 adult (not maiden) twin-bearing Merino ewes mated to Merino rams were used. The ewes were randomly allocated to four groups and grazed the lambing paddocks, or similar pasture, for between 0 and 31 days prior to the start of lambing. Variation in time was due to some producers delaying entry into the lambing paddock so as to save available pasture in those paddocks for the lambing period. The lambing paddocks were subdivided to provide blocking for treatments. A description of the experimental sites is shown in Table 1.

At each experimental site, a random sample of 50 ewes per paddock were condition-scored (Jefferies 1961) between 6 and 10 days before the start of the lambing period. At this time, blood samples were collected from 10 ewes per paddock by using 9 mL lithium heparin vacutainers. The samples were stored on ice prior to being centrifuged, and the plasma was separated and frozen at −20°C until analysis. At the same time, urine samples were collected from the same 10 ewes by nasal occlusion (Benech *et al.* 2015). Urine pH was

Table 1. Description of experimental sites and mean pasture availability, supplement intake, ewe mortality and lamb survival for Experiment 1 in 2017.

Item	Tullamore	Pingelly	Kingston	Robe	Holbrook
State	NSW	WA	SA	SA	NSW
Lambing start	9 Jul	20 Jul	1 Sep	1 Sep	17 Aug
Number of ewes	450	400	400	400	600
Lambing paddock size (ha)	60	15–18	21	22–30	10
Pasture type	Barley grass/clover	Capeweed/subclover	Phalaris/annual grass/clover	Phalaris based	Phalaris/clover/annual grass
Grass (%)	64	26	42	68	95
Live pasture pre-lambing (kg DM/ha)	455 ± 60	432 ± 25	1868 ± 123	1308 ± 86	2830 ± 297
Supplement intake (g/day)	19.0	12.6	0.4	2.0	8.1
Ewe mortality (%)	1.8	3.5	4.7	4.2	2.0
Lamb survival (%)	80	59	51	68	67

measured immediately using a pH meter (Pingelly: Thermo Scientific Orion Star A325; SA: TPS WP-80; Holbrook: Model pH700, Eutech instruments, Singapore), and specific gravity was measured using a refractometer (Tullamore, Pingelly and SA: clinical urine refractometer TE-RM200SGRI, Testequip; Holbrook: FG302/312 portable refractometer, Australian Instrument Services, Melbourne, Vic., Australia). Urine samples were then stored on ice for transport, and frozen at −20°C until analysis. After sampling, each group of ewes was placed into a lambing paddock (10–60 ha), and mineral supplementation was commenced. Post-lambing blood and urine samples were not collected because previous studies (Masters *et al.* 2019) have shown that a similar type and method of supplementation improved the mineral status of late pregnant ewes.

The loose lick of minerals was offered to the supplemented groups from the day of pre-lambing sampling until the end of the lambing period. The minerals were fed in troughs twice weekly, with refusals collected once weekly and weighed to estimate intake. If the refusals were wet, subsamples were weighed, dried, and re-weighed to calculate dry weights. Due to the low quantity of available pasture, the ewes at Tullamore were fed oaten grain at 0.5 kg/ewe per day throughout the lambing period. Ewes at Pingelly and the other locations were not supplementary fed grain or hay.

Approximately 2 weeks after the end of lambing, condition score was assessed on the same 50 ewes that were assessed pre-lambing. Percentage lamb survival was calculated per paddock as the number of lambs present at marking age/the number of twin fetuses allocated to each paddock. Postmortems were not conducted on ewes or lambs that died during the study.

The quantity of live and dead pasture was estimated from 10 quadrat cuts per paddock, both at the time of blood sampling pre-lambing, and at lamb marking. Pasture cuts were sorted into components, dried at 60°C, then weighed. Grab samples were collected at the same time for analysis of mineral content, and dried at 60°C prior to laboratory analyses.

Laboratory analyses

Pasture grab samples, plasma and urine samples were analysed only for the Tullamore and Pingelly sites because mineral intake at the other locations was negligible. The grab samples were sent to a commercial laboratory (CSBP Soil and Plant Analysis Laboratory, Bibra Lake, WA, Australia) for standard mineral analyses. The percentages of minerals in the pasture DM were used to calculate the Ca:P ratio, K:Na ratio and tetany index = (K/0.039)/((Mg/0.012) + (Ca/0.02)), and were converted to molecular equivalents/100 g DM to calculate DCAD = (Na/0.023 + K/0.039) − (Cl/0.0355 + S/0.016; Masters *et al.* 2019). The concentrations of Mg, Ca and phosphorus (P) in plasma and urine samples were analysed using kits (photometric colour-test method). Plasma and urine creatinine was analysed using a creatinine BLOSRx78 kit (kinetic colour Jaffe method) and a Beckman Coulter AU480 analyser (Beckman Coulter Ltd, UK; Veterinary Diagnostic Laboratory, Charles Sturt University, NSW, Australia). Fractional excretion of each mineral was calculated as (concentration in urine × plasma concentration of creatinine)/(plasma concentration × urine concentration of creatinine), converted to percentage (Bhanugopan *et al.* 2015).

Statistical analyses

Genstat software, 18th edition (VSN International, Hemel Hempstead, UK) was used for statistical analyses. Only data for Tullamore and Pingelly were analysed due to negligible supplement intake at the other locations. Live pasture availability pre- and post-lambing was transformed by square root prior to analyses by using linear mixed models with location × treatment as the fixed effect and location × replicate as the random term. Plot means for lamb survival and ewe condition score were analysed using the same model. Plasma and urine data were analysed with similar models by using individual ewe data, with the exceptions of urine specific gravity and that urine pH was only available for the Pingelly location, and an exponential

transformation was used for this variable prior to analysis. One outlier (4.38%, 2.7 times the standard deviation) was removed from the Control treatment for analysis of fractional excretion of Ca. Urine-specific gravity was analysed using the non-parametric Wilcoxon rank-sum test to compare treatments and locations. The mineral concentrations in pasture pre- and post-lambing were also analysed using the non-parametric Wilcoxon rank-sum test. Differences were considered significant if $P \leq 0.05$.

Experiment 2

This study was conducted to repeat the 2017 study with the intention of achieving target mineral intake, and to compare two forms of Ca and Mg supplement [standard (Causmag/lime) and low-DCAD]. The study was conducted during 2018 at the Charles Sturt University commercial farm, Wagga Wagga, NSW, Australia. A randomised design without blocking was used, as paddock size prevented subdivision of all paddocks into full replicates. However, all lambing plots used were adjacent, and based on lucerne (*Medicago sativa*) pasture. All plots were between 19 and 23.5 ha in size.

The design comprised two replicates of the following three treatments: non-supplemented Control; Standard: a loose mix of lime, Causmag (calcined MgO) and salt in the ratio by weight of 1:1:1; and Low-DCAD: a low cation–anion difference (DCAD), comprising Mg chloride, Ca sulfate and salt in the ratio 12.5:32.5:55.0, as used in the 2017 study. Both supplements were manufactured by DSM Nutritional Products, Australia, and the low-DCAD supplement was the same batch as used in Experiment 1.

Sheep management and measurements

Twin-bearing, mature composite ewes ($n = 600$) joined to composite rams and due to lamb over 6 weeks from 6 July were used. On Day 120 from the start of joining, blood and urine samples were collected and processed from 90 randomly selected ewes, using the same methods as in Experiment 1. The day after sampling, the ewes were moved to the experimental site and randomly divided into six groups ($n = 100$/group), ensuring 15 sampled ewes per group. A random sample of 50 ewes per group was condition-scored (Jefferies 1961) before each group was randomly allocated to a lambing plot. The same ewes were re-sampled on Day 140 after the start of joining. Due to low pasture availability, the ewes were supplementary fed cereal grain (wheat and barley) while on the lambing plots, commencing at a rate of 0.8 kg/ewe per day, and increasing to 1.5.

Mineral supplements were offered from 9 June, 123 days after the start of the 6-week joining. The supplements were fed in troughs, at a rate of 30 g/ewe per day for 13 days, after which the rate was reduced to 20 g/ewe per day due to excess refusals. The supplement was fed until 3 August, 136 days after the end of joining, when it was estimated that 90% of ewes had lambed. The minerals were fed each 1–2 days for the first week of feeding, after which they were fed every 3–4 days. Mineral intake was calculated from the refusals which were dried, if necessary, before weighing.

Lamb liveweight and survival to marking age were recorded on 13 August when the youngest lambs were a week old. Plot means for survival were calculated as the percentage of lambs present at marking of the number of fetuses previously allocated to each lambing plot. Ewe condition score was again recorded for the same ewes as sampled previously. The number of ewe mortalities for each plot was recorded during the lambing period. However, postmortems were not conducted on either ewes or lambs that died during the study.

Live pasture availability was visually estimated in 100 quadrats (0.1 m^2) per plot on 14 June, when ewes were in late pregnancy, and 14 August at the end of lambing, with calibration against 20 quadrats (Haydock and Shaw 1975). The calibration quadrats were estimated, cut with electric clippers and dried at 60°C, before weighing. Grab samples of live herbage were also collected from each plot on 14 June, prior to the start of lambing, and analysed for mineral content as in Experiment 1.

Laboratory analyses

The concentration of Mg, Ca and P in plasma and urine samples was analysed using an inductively coupled plasma–emission spectrophotometer (Environmental and Analytical Laboratories, Charles Sturt University, Wagga Wagga, NSW, Australia). Plasma and urine creatinine was analysed and fractional excretion of each mineral was calculated as for Experiment 1. Technical error required the samples to be re-analysed, and insufficient sample was available for some ewes, so that samples could be re-tested only for between 5 and 12 ewes in each of the six lambing groups.

Statistical analyses

Data were analysed using Genstat software. Ewes were excluded from plasma and urine analyses if they were not sampled on both occasions. Urine and plasma variables and ewe condition score were analysed using linear mixed models with day of sampling × treatment as the fixed effect and plot or plot + plot.ewe as the random term. Data for fractional excretion of Ca, Mg and P were transformed by natural logarithm prior to analysis to equalise variances. The weight of lambs and percentage lamb survival were also analysed using linear mixed models, with treatment as the fixed and plot as the random term. Plot means for the quantity of live pasture were analysed using linear mixed models with time × treatment as the fixed and plot as the random term. The mineral concentration in herbage was analysed using a non-parametric Wilcoxon rank-sum test. Differences were considered significant if $P \leq 0.05$.

Results

Experiment 1

Pastures, supplement and production

The total (live plus dead) quantity of pasture was similar between mineral treatment groups within locations. There were negligible quantities (<20 kg DM/ha) of dead pasture at Pingelly, Robe, and Kingston pre-lambing, but Holbrook and Tullamore pastures contained large quantities of dead material, typically 1000–2000 kg DM/ha. The mean quantity of live pasture pre-lambing varied widely between sites (Table 1), but for the two sites analysed, it differed between treatments only at Tullamore post-lambing (Table 2). Intake of the low-DCAD mineral supplement was negligible at Kingston, Robe and Holbrook, locations where live pasture availability was high pre-lambing. Supplement intake was higher at Tullamore and Pingelly, but below the target quantity of 30 g/ewe per day (Table 1).

Ewe condition was 0.2 score higher at Tullamore than at Pingelly pre- but not post-lambing (Table 2), and was similar between treatments pre-lambing (3.1 ± 0.03 and 3.1 ± 0.03) and post-lambing (2.9 ± 0.04 and 2.9 ± 0.04). The survival of lambs to marking age varied widely between locations (Table 1) but varied by less than 3% between treatments at all locations except Tullamore. For the two sites where supplement intake was achieved, lamb survival was not increased by mineral supplementation in the form of low-DCAD (Control 64.5 ± 1.04%; Low-DCAD 64.9 ± 1.04%; Table 2). The mortality of ewes during the lambing period (range 1.8–4.7%; Table 1) was similar between treatments within location (data not shown).

Pasture and plasma mineral concentrations and fractional excretion

The concentration of Ca, Mg, P, K and Na in grab samples of pasture from Tullamore and Pingelly were similar between treatments (Table 3) and generally at or above required concentrations (Hocking Edwards *et al.* 2018), with the exception of P at Tullamore. However, there were large differences in mineral concentration between pastures at the two locations, with Tullamore showing lower concentrations of Ca, Mg, P and Na than did Pingelly. Pastures at both sites exhibited high DCAD levels.

Plasma concentrations of Ca, Mg and P, urine pH and fractional excretion of Mg were similar between treatments prior to supplementation (Table 3), as expected, for the Tullamore and Pingelly ewes. Mg concentrations in plasma were similar (P = 0.062) between treatments (Control 21.5 ± 0.56 mg/L; Low-DCAD 22.1 ± 0.56 mg/L). Fractional excretion of Ca showed an interaction between location and treatment, due to higher rates in Control ewes at Tullamore.

Plasma Mg concentrations were adequate in most ewes at both locations. However, only 55% of Pingelly ewes had adequate Ca concentrations in plasma (Table 4), although the deficiency was not severe as only 10% of the ewes had 80 mg/L or less. At Tullamore, the majority of ewes showed deficient concentrations of plasma P. The fractional excretion of Ca and Mg was similar between treatments and locations. However, the fractional excretion of Ca was lower in the Low-DCAD treatment at Tullamore, but not at Pingelly.

Experiment 2

Pastures, supplement and production

The quantity of live pasture before and after lambing was similar between treatments and there was no interaction between time and treatment (Table 5). The percentage mineral concentration of the pasture was similar between treatments, with Ca 2.7 ± 0.03, Mg 0.4 ± 0.0, P 0.2 ± 0.0, K 1.9 ± 0.1, Na 0.2 ± 0.1% and generally at or above the required levels (Hocking Edwards *et al.* 2018). One of the Low-DCAD treatment plots exhibited a high DCAD, raising the mean for that treatment. The percentage mineral

Table 2. Mean live-pasture availability (kg DM/ha), ewe condition score pre- and post-lambing, and lamb survival (%) at two locations in Experiment 1 in 2017.

Item	Time	Treatment	Tullamore	Pingelly	*P*-value		
					L	Trt	L × Trt
Live pasture (kg DM/ha)[A]	Pre-lambing	Mean[B]	455 ± 60	432 ± 25	0.921	0.802	0.462
	Post-lambing	Control	476 ± 78	1414 ± 118	0.014	0.010	0.004
		Supplement	152 ± 17	1304 ± 52			
Condition score	Pre-lambing	Mean	3.2 ± 0.02	3.0 ± 0.02	<0.001	0.944	0.944
	Post-lambing	Mean	2.9 ± 0.04	2.9 ± 0.04	0.709	0.224	0.224
Lamb survival (%)		Control	82.7 ± 1.95	58.3 ± 1.95	<0.001	0.26	0.077
		Supplement	77.0 ± 1.95	59.5 ± 1.95			

P-values are given for location (L), treatment (Trt) and their interaction.
[A]Pasture means are raw values.
[B]Overall means are shown when treatment means were similar ($P > 0.05$).

Table 3. Mean ± s.e.m. urine and plasma variables pre-lambing, prior to supplementation, for Control and mineral-supplemented ewes, and mean pre- and post-lambing mineral concentration of pastures at Tullamore and Pingelly in Experiment 1 in 2017.

Item	Treatment	Tullamore	Pingelly	*P*-value		
				Trt	L	Trt × L
Urine pH[A]	Mean	–	7.43 ± 0.119	0.562	–	–
Specific gravity[A]	Mean	1.33 ± 0.001	1.34 ± 0.000	0.611	**0.006**	–
Ca plasma (mg/L)[A]	Mean	95.36 ± 0.815	90.30 ± 1.397	0.673	**0.004**	–
Mg plasma (mg/L)	Mean	22.06 ± 0.555	21.51 ± 0.555	0.062	0.773	0.118
P plasma (mg/L)	Mean	32.24 ± 2.83	56.22 ± 2.83	0.445	0.109	0.653
FE Ca (%)	Control	1.16 ± 0.123	0.60 ± 0.120	0.226	0.294	**0.009**
	Supplement	0.66 ± 0.120	0.78 ± 0.120			
FE Mg (%)[A]	Mean	23.77 ± 1.291	21.05 ± 1.291	0.078	0.574	0.355
Pasture Ca (%)[A]	Mean	0.55 ± 0.031	1.20 ± 0.075	0.367	**<0.001**	–
Pasture Mg (%)[A]	Mean	0.24 ± 0.006	0.28 ± 0.005	0.421	**0.002**	–
Pasture P (%)[A]	Mean	0.19 ± 0.014	0.37 ± 0.033	0.939	**<0.001**	–
Pasture K (%)[A]	Mean	2.24 ± 0.122	2.07 ± 0.254	0.342	0.382	–
Pasture Na (%)[A]	Mean	0.09 ± 0.026	0.93 ± 0.077	0.290	**<0.001**	–
Pasture Ca:P[A,B]		2.39 ± 0.317	2.66 ± 0.278	0.279	0.505	–
Pasture K:Na[A,C]		34.18 ± 17.070	1.39 ± 0.244	0.505	**<0.001**	–
Pasture K:Na + Mg[A,D]		2.44 ± 0.223	0.86 ± 0.136	0.959	**<0.001**	–
Pasture DCAD[A,E]		27.36 ± 1.218	29.24 ± 2.612	**0.038**	0.959	–
Pasture Tetany index[A,F]		1.22 ± 0.097	0.65 ± 0.086	1.000	**0.002**	–

P-values are given for location (L), treatment (Trt) and their interaction ($P < 0.05$ values in bold).
[A]Raw means.
[B]Required ratio of Ca:P 1.1–2:1.
[C]Required ratio of K:Na 5.6–7.1 as cited by (CSIRO 2007).
[D]Required ratio of K:Na + Mg <6.
[E]Required level of DCAD <12.
[F]Required level Tetany index <2.20, as cited by Hocking Edwards *et al.* (2018).
Ca, calcium; Mg, magnesium; P, phosphorus; K, potassium; Na, sodium; FE, fractional excretion.

Table 4. Percentage of ewes with plasma mineral concentrations within the adequate range pre-lambing and prior to supplementation in Experiment 1 in 2017.

Item	Tullamore	Pingelly
Ca plasma >90 mg/L (%)[A]	85	55
Mg plasma >18 mg/L (%)[A]	100	90
P plasma >45 mg/L (%)[B]	15	90

[A]Suttle (2010).
[B]Constable *et al.* (2017).

content of the grain fed during the experiment was Ca 0.06–0.07, Mg 0.13–0.18, P 0.3–0.43, K 0.4, and Na <0.01%, indicating deficiency in Ca and Na. Both of the mineral supplements were palatable with the refusals, indicating *ad libitum* intake throughout most of the feeding period, and intake approaching the target level.

The condition score of ewes did not vary with treatment. Condition score declined from Day 120 (3.1 ± 0.03) to Day 140 and post-lambing (3.0 ± 0.03). Lamb survival and weight at marking age were not increased by mineral supplementation (Table 5). Throughout the lambing period, 14 (2.3%) ewes died, with a similar mortality rate among treatments, namely, 2% in each of Control and Low-DCAD, 3% in the Standard treatment.

Plasma mineral concentrations and fractional excretion

Plasma and urine mineral concentrations are shown in Table 6. Supplementation did not increase the proportion of ewes with adequate plasma Ca, Mg or P concentrations. Mean Ca and Mg concentrations in plasma increased between Day 120 and Day 140, but were similar among treatments. Few ewes (<5%) had adequate Ca concentrations (>90 mg/L) at Day 120, prior to mineral supplementation, and while the concentrations had increased by Day 140, only 24–61% had adequate concentrations at this time. Mg concentrations were adequate (>18 mg/L) in approximately half of ewes in all treatments at Day 120, but by Day 140, the majority had adequate concentrations.

Table 5. Mean supplement intake, live pasture available pre- and post-lambing, lamb liveweight and survival to marking age from three mineral-supplementation treatments of Experiment 2 at Wagga Wagga in 2018.

Item	Control	Low-DCAD	Standard	s.e.m.	*P*-value
Mean supplement intake per ewe (g/day)	0	19.6	18.2		–
Live pasture available pre-lambing (kg DM/ha)	594	367	867	232	0.630
Live pasture available post-lambing (kg DM/ha)	568	554	661	232	
Lamb survival to marking (%)	77	77	77	3.8	0.999
Mean lamb weight at marking (kg)	12.7	12.9	13.7	0.60	0.542

Table 6. Plasma and urine calcium (Ca), magnesium (Mg) and phosphorus (P) concentrations, urinary fractional excretion (FE) and urine pH from three mineral-supplementation treatments in Experiment 2 on Days 120 and 140 from joining at Wagga Wagga in 2018.

Item	Day	Control	Low-DCAD	Standard	s.e.m.	*P*-value		
						Trt	Day	Trt × Day
Plasma Ca (mg/L)	120	78.93	79.16	79.62	1.727–1.863	0.570	<0.001	0.618
	140	88.17	92.02	90.84				
Proportion of ewes with Ca >90 mg/L	120	0.00	0.05	0.05		0.082	<0.001	0.711
	140	0.39	0.76	0.47				
Plasma Mg (mg/L)	120	17.76	18.74	18.81	0.713–0.786	0.390	<0.001	0.928
	140	21.88	23.04	22.47				
Proportion of ewes with Mg >18 mg/L	120	0.53	0.63	0.60		0.583	0.020	0.457
	140	0.83	0.94	0.74				
Plasma P (mg/L)	120	73.20	72.41	75.68	2.786–3.022	0.833	0.058	0.215
	140	69.04	72.98	66.10				
FE Ca (%)[A]	120	0.04	0.02	0.03	0.006–0.019	0.520	0.023	0.637
	140	0.14	0.15	0.05				
FE Mg (%)[A]	120	0.41	0.52	0.56	0.086–1.161	0.867	<0.001	0.599
	140	2.00	3.18	2.00				
FE P (%)[A]	120	0.01	0.01	0.01	0.001–0.019	0.167	<0.001	0.071
	140	0.02	0.06	0.02				
Urine pH	120	6.70	6.87	6.80	0.146–0.159	0.663	<0.001	0.190
	140	8.26	7.84	8.07				
Pasture Ca:P[A]		12.30	6.86	9.24	0.316–2.223	>0.05		
Pasture K:Na[A]		9.53	4.99	6.88	0.145–2.708	>0.05		
Pasture K:Na + Mg[A,B]		1.37	1.22	1.48	0.053–0.383	>0.05		
Pasture DCAD[A,C]		5.09	17.77	11.93	1.392–7.668	>0.05		
Pasture Tetany index[A,D]		0.25	0.42	0.27	0.009–0.004	>0.05		

P-values are given for treatment (Trt), day of sampling (Day) and their interaction (Trt × Day).
[A]Raw means, data transformed for analysis.
[B]Required level <6.
[C]Required level <12.
[D]Required level <2.20, as cited by Hocking Edwards *et al.* (2018).

Urine pH increased between Day 120 (6.79 ± 0.093) and Day 140 (8.05 ± 0.093), but was similar among treatments. Supplementation did not change the fractional excretion of Ca, Mg or P, although the percentage excretion increased between Days 120 and 140.

Discussion

This study showed no improvement in lamb survival as a result of offering loose-lick Ca and Mg supplements to grazing ewes during the lambing period at the industry

recommended rate and with target supplement intake. Insufficient supplement intake during Experiment 1 contributed to the lack of benefit, with three of the five sites failing to achieve meaningful levels of intake. This highlights a need for further studies to clarify the conditions under which adequate voluntary supplement intake may be achieved under grazing conditions, and means of increasing intake.

The variation in supplement intake observed between sites may be associated with the type and quantity of pasture grazed. Lucerne is a known natrophobe with low accumulation of Na in leaf, particularly under dryland conditions (Hall 1982; Champness *et al*. 2021), so salt-based supplements may be attractive to sheep grazing lucerne, consistent with the intake recorded in Experiment 2. High intake of the same low-DCAD supplement (21–30 g/day) has been reported previously for ewes grazing cereal crops, which are also often deficient in Na, although more variable intake (mean 22, range 4.9–30 g/day) was recorded for a lime/Causmag/salt loose lick (Masters *et al*. 2019). Alternatively, supplement intake may be reduced where high quantities of live pasture are present, since intake was minimal at three sites in Experiment 1 when the quantity of live pasture was ≥1300 kg DM/ha. This is consistent with a previous report where loose-lick intake declined following germination of forage (White *et al*. 1992). In our study, a low palatability of the supplement may also have contributed to low intake, as observations indicated high refusals when the supplement became wet from either rain or from absorption of moisture from the air. Further work is needed to identify the grazing and climatic situations that promote high supplement intake.

Variability in intake among ewes may have contributed to the lack of effect of group supplementation in the present study, even when target levels of mean intake were achieved. Supplementation of grazing ewes with Mg oxide (Causmag) at a rate of 14 g/ewe every second day, by spraying onto hay or mixing with grain, has prevented hypomagnesaemia in deficient flocks (Herd 1966). However, those methods of supplementation may achieve more uniform mineral intake than free-choice forms of supplementation such as a loose lick. Variation in free-choice supplement intake among ewes is known to be large (Bowman and Sowell 1997), with a proportion of ewes consuming no supplement being affected by factors such as neophobia, self-feeding and limited trough space. There is large variability among sheep in intake of block or liquid supplements (coefficient of variation <100%) (Mulholland and Coombe 1979) and of loose-lick mineral supplements (1.6-fold) (White *et al*. 1992). Despite the variable intake, loose-lick mineral supplementation has increased weaner weight gain (White *et al*. 1992) and, elsewhere, plasma Ca and Mg concentrations in late-gestation ewes (Masters *et al*. 2019). These indicate that variable intake among ewes should not prevent a response in lamb survival if the response to supplementation is large, unless intake is low or few ewes consume the supplement. The variability of intake among individuals in the present study is unknown, but may have contributed to the lack of influence on metabolic mineral concentrations.

Importantly, lamb survival was also not increased where an average target intake of 20 g/day of supplement was achieved in the present study. There was no indication from the ewe mortality rates that Ca and Mg supplementation reduced the risk of ewe death or dystocia, which is known to occur in clinically Ca-deficient ewes due to reduced uterine activity (Robalo Silva and Noakes 1984). Likewise, although supplementation has improved the immunity status of lambs (Ataollahi *et al*. 2020), and may reduce the duration of parturition even in ewes with adequate Ca and Mg concentrations (Ataollahi *et al*. 2021), if these occurred in the current study, they did not increase lamb survival. Lambs that survive a difficult birth have a reduced ability to regulate body temperature (Darwish and Ashmawy 2011), and an increased latency to suckle (Dwyer 2003; Dutra and Banchero 2011), both of which make them more susceptible to hypothermia. The winter lambing, which occurred in Experiment 2, provided cold conditions (average minimum temperature 3.2 ± 3.2°C; Bureau of Meterology, www.bom.com) where mineral supplementation, if it reduces the duration of parturition, might be expected to demonstrate an increase in lamb survival as a result of better temperature regulation. However, no benefit in survival was apparent despite target mean supplement intake being achieved. This is consistent with a report for ewes grazing cereal forage deficient in Ca and Mg, with supplement intake of 24 g/day (McGrath *et al*. 2015). The present study highlighted the variation between locations (51–83%) in survival of twin lambs, which is consistent with previous literature (Kleemann and Walker 2005). Factors other than Ca and Mg deficiencies need to be investigated, particularly at those sites where survival was poor.

Ca and Mg supplementation from 5 weeks before lambing to 4 weeks of age in pen-fed ewes has previously been shown to increase lamb growth rates up to 4 weeks of age (Ataollahi *et al*. 2020), despite non-supplemented ewes having adequate mineral concentrations. Ca and Mg supplementation of subclinically deficient ewes in Experiment 2 did not indicate any effect of supplementation on lamb weight. It is possible that the level of deficiency in Experiment 1 or 2 was not sufficient to affect the rate of dystocia, lamb survival or lamb growth rate to marking age. However, since positive effects on duration of parturition, growth and immunity status have been recorded in pen-fed ewes with adequate mineral concentrations (Ataollahi *et al*. 2020, 2021), our results may also indicate that, under grazing conditions, these effects are too small, are not the key factors in perinatal lamb survival, and/or variation in supplement intake prevented any production benefit.

The degree of mineral deficiency in non-supplemented ewes may have contributed to the effectiveness of

supplementation. The level of supplement intake at the Tullamore site (19 g/ewe per day) in Experiment 1 might be expected to be sufficient to raise plasma and urine mineral concentrations (Masters *et al.* 2019), although a similar level of intake did not appear to improve the mineral status of ewes in Experiment 2. However, at Tullamore prior to lambing and supplementation, only 15% of ewes showed subclinically deficient Ca concentrations, and none was Mg deficient, so it is probable that any increase in metabolic concentrations would be ineffective for increasing lamb survival. The P deficiency measured in most Tullamore ewes may have contributed to the risk of hypocalcaemia at that site (SCA 1990), but no cases were reported and ewe mortality was low. In addition, the 83% survival rate for Control lambs was above the industry average of 70% for twins (Hinch and Brien 2014). As a result, the potential for mineral supplementation to further increase survival was reduced in this flock. The lower quantity of live pasture for the supplemented treatment post-lambing probably reflects sampling error and difficulty in sorting live from dead in dried samples, rather than large actual differences in pasture availability that may have influenced survival.

In contrast, nearly half of the ewes at Pingelly in Experiment 1 were subclinically deficient in Ca. It is possible that the level of supplement intake (12.6 g/ewe per day) at this location may not have been adequate to affect the mechanisms involved in lamb survival, but blood samples were not collected post-lambing so as to determine any change in metabolic levels. The type of pasture grazed may also have reduced any benefit from supplement intake. Clover generally has higher concentrations of Ca and Mg than do grass species (Metson and Saunders 1978), although when grazing mixed pastures, many ewes may still become subclinically deficient by late pregnancy (Hocking Edwards *et al.* 2018).

In Experiment 2, nearly all ewes were deficient in Ca at Day 120, prior to supplementation. This may have been due to the ewes having been sustained on a cereal grain-based ration prior to that point. Cereal grain is deficient in Ca, and could be expected to contribute to deficiency in late pregnant/lactating ewes (SCA 1990). Although the ewes grazed a legume-based pasture during late pregnancy, they also required a cereal grain ration due to insufficient quantity of pasture. The lucerne pasture had a high K:N ratio, which affects both Na and Mg absorption, warranting supplementation with Na and Mg in young sheep (Dove and McMullen 2009). These factors indicate a situation where Ca and Mg supplementation could be expected to be effective if subclinical deficiency reduces lamb survival. However, despite near target intake of mineral supplement in Experiment 2, 24–53% of ewes were still subclinically deficient in Ca at Day 140, prior to lambing, so neither supplement appeared to alter the mineral status of ewes, and neither supplement improved lamb survival.

A change in plasma Ca concentrations would not be expected because these are under homeostatic control mechanisms (Goff 2000). Supplementation at a similar level has previously been shown to have an inconsistent impact on plasma and urine Mg concentrations in grazing ewes (Masters *et al.* 2019), although it has increased concentrations in pen-fed ewes (Ataollahi *et al.* 2018). In contrast, Ca status was expected to improve on the basis of previous grazing studies (Masters *et al.* 2019). In the present report, Experiment 2 used a lower ratio of lime, Causmag and salt (1:1:1 by weight) than did earlier studies (2:2:1; Masters *et al.* 2019), so that the lower mineral concentration may have reduced its effectiveness. However, the lack of difference in fractional excretion of Ca or Mg between the low-DCAD, standard and non-supplemented treatments in Experiment 2 indicated that the 1:1:1 loose mix was not less effective than the low-DCAD form. These contrasting results indicated that it may be difficult to predict whether supplementation will improve the mineral status of ewes.

The reduction in the proportion of ewes subclinically deficient in Ca or Mg during late pregnancy in Experiment 2, regardless of supplementation, was unexpected, and may reflect an improvement in mineral status after changing from a grain-based diet to one including green lucerne pasture. It is unknown whether this prevented a benefit from supplementation, despite a proportion of ewes being deficient in Ca and Mg pre-lambing. It is also unknown whether offering a higher level of supplement would have altered the mineral status of ewes or lamb survival in either study.

Conclusions

Offering a Ca and Mg loose-lick supplement, including in a low-DCAD form, to subclinically deficient grazing ewes at recommended rates did not increase perinatal lamb or ewe survival at any of the sites in this study, nor did it improve the Ca or Mg status in the flock where this was monitored. Further studies are required to establish whether there is any benefit at greater degrees of subclinical deficiency, at higher levels of supplementation or in different conditions before clear recommendations can be given.

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Author affiliations

[A]School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Boorooma Street, Wagga Wagga, NSW 2678, Australia.

[B]Gulbali Institute, Charles Sturt University, Boorooma Street, Wagga Wagga, NSW 2678, Australia.

[C]Fred Morley Centre, Charles Sturt University, Boorooma Street, Wagga Wagga, NSW 2678, Australia.

[D]South Australian Research and Development Institute, Struan Research Centre, Penola Road, Naracoorte, SA 5271, Australia.

[E]Centre for Production Animals and Health, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia.

[F]New South Wales Department of Primary Industries, Cowra Agricultural Research and Advisory Station, Binni Creek Road, Cowra, NSW 2794, Australia.

[G]Charles Sturt University, Boorooma Street, Wagga Wagga, NSW 2678, Australia.