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Fail safe guides to pregnant and lambing ewes on cereals – Phase II

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Abstract

Grazing vegetative cereal crops is becoming an important strategic and tactical grazing option on mixed farms in Australia. However, metabolic disorders have been reported and many producers avoid grazing crops with reproducing ewes, others accept the risk and losses that go with it. This project aimed to define the causes of metabolic risk and investigate options to minimise the risk. Mineral status of vegetative crops (wheat, barley and oats) and pregnant ewes grazing these crops was monitored on 18 farms in WA and NSW. Responses to supplements on six farms were also measured. Crops (particularly wheat grown in NSW) provided ewes with an unbalanced mineral supply of low Ca, Mg and Na accompanied by high K. This combination increases the risk of hypocalcaemia and hypomagnesaemia. Partially consistent with this observation, ewes grazing crops had a low Ca status but did not show clear signs of low Mg status. Providing supplements containing Ca, Na and Mg improved the mineral status of the ewes and should be considered as a risk management strategy. Given that both endocrinological change and Ca status contribute to the onset of hypocalcaemia some evaluation of supplements under conditions of acute Ca stress are required.

Executive summary

Grazing vegetative wheat, barley and oats (both dual purpose and traditional spring varieties) is becoming an important strategic and tactical grazing option on farms where crops and livestock coexist. Surveys of producers and consultants indicate 15 to 60% of farmers with sheep and crops have adopted this practice. The high winter growth rates of the crops offer the opportunity to fill the winter feed gap and the high nutritive value (metabolisable energy and protein) make these crops well suited to meet the requirements of reproducing ewes. However, metabolic disorders have been reported and many producers avoid grazing these crops with reproducing ewes, others accept the risk even though they have encountered metabolic disorders. This project aimed to define the risk and develop supplements and strategies that would minimise ewe mortality and ill health. This was addressed through a two-year experimental program.

In the first year the mineral status of both forage and of reproducing ewes grazing wheat, oats or barley was monitored on 18 farms. The farms were located in Western Australia (6 farms) southern New South Wales (7 farms) and central New South Wales (5 farms). Crops grazed were wheat (8 farms), barley (4 farms) or oats (6 farms) and the average period of grazing was 20 days. Samples of blood and urine were collected pre and post grazing from ewes and samples of crop and soils also collected for mineral analysis. A high proportion of farms had forage Ca (70%), Na (70%), Mg (18%) below published requirements and K (70%) above the published Maximum Tolerable Level. Analysis of samples collected from the ewes at the end of crop grazing indicated ewes on 94% of farms had alkaline urine and on 88% of farms Ca concentrations in the urine were in the marginal range. In conclusion, the forages had a complex mineral composition meaning grazing ewes had an increased risk of direct or induced Ca (hypocalcaemia) or Mg (hypomagnesaemia) deficiency. Preliminary analysis indicated higher risks from grazing wheat and/or grazing crops grown on high K soils.

In year 2 the effectiveness of two mineral supplements was assessed on six farms over three weeks. One of these was a standard industry supplement containing causmag, limestone, and salt (40:40:20), the other contained the same cations but as magnesium chloride, gypsum and salt (12.5:32.5:55). The second supplement was designed to reduce the dietary cation-anion difference in the ingested diet. Both supplements were provided *ad libitum* up to 30 g/day. On each farm 90 twin bearing ewes in late gestation (d115 to d129 of gestation) were divided into 3 treatment groups (n=30/treatment). One group of ewes were a control group and given no supplement a second group were provided with the industry standard and the third group was provided with the new supplement. The ewes were rotated around the three plots each week. Samples of blood and urine were collected pre and post grazing from ewes and samples of crop and soils also collected for mineral analysis. The supplemented ewes showed significant increases in Ca concentration in urine, plasma and Ca fractional excretion on all but one of the six farms. There were no clear or consistent differences between the two supplemented groups of ewes. It was concluded that the Ca status of ewes grazing vegetative cereal crops in late pregnancy can be improved by providing supplements containing Ca, Mg and Na. No production responses to the supplements were apparent, however this was not unexpected as large commercial scale experimentation is required to detect changes in ewe mortality.

Integration of results from the two years indicated that:

1. Crop forage, even at FOO < 200 kg DM/ha was adequate to maintain or improve condition score in late-pregnant ewes;
2. Low and unbalanced supply of minerals in forage and low or marginal status of Ca in body fluids indicates ewes have an increased risk of direct or induced Ca while grazing young crops in late pregnancy;
3. There was no strong evidence of a risk of clinical Mg deficiency, but low Mg status may predispose ewes to hypocalcaemia;
4. Risks of imbalance was highest for wheat growing in NSW followed by oats with lowest risk in grazing barley. As not all crops were grown in all locations further crop analysis is required to define the interaction between crop type and location.
5. Ca status is improved by providing supplements containing Ca, Mg and Na
6. Provision of a supplement containing Ca may improve status and reduce risk of deficiency but requires testing under acute Ca stress where an interaction between Ca status and endocrinological change will influence the incidence of hypocalcaemia.

Key messages that have resulted from this research and the review of literature on crop grazing are:

1. In dry or drought periods where breeding ewes are supplemented with grain, Ca supplements should also be provided. High grain diets with a lack of Ca may result in Ca depletion and increased susceptibility to hypocalcaemia when grazing crops at a later time.
2. Young vegetative crops are an excellent source of metabolisable energy (approximately 12 MJ/kg DM) and protein (crude protein 15 to 37 %) for pregnant ewes;
3. The forage is available at a time when nutrient requirements of reproducing ewes is high and ewes can maintain condition score while grazing forages with feed on offer of <200kg DM/ha. This is a much lower FOO than is required when grazing conventional pastures;
4. Forage provides an unbalanced supply of minerals to grazing ewes with low Na, marginal Ca and Mg and high K. This imbalance results in low Ca status and marginal Mg status and an increased risk of metabolic disorders such as hypocalcaemia, hypomagnesaemia and pregnancy toxaemia;
5. Mineral supplements will increase Ca and to a lesser extent Mg status. It remains to be confirmed that such supplements will overcome the endocrinological disturbance that causes hypocalcaemia under conditions of acute Ca stress. Supplements require testing on a commercial scale;
6. Any mineral supplement used should contain Ca, Mg and Na;
7. Wheat crops grown in NSW are high risk forage. It is likely but not yet confirmed that wheat crops elsewhere will also be high risk. Sheep grazing oats and barley are also consuming an unbalanced mineral supply and should not be considered risk free.

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1 Background

Increasing the area of cereal crops grazed by pregnant and lactating ewes during winter represents a major opportunity to increase sheep numbers in the wheat-sheep zone, where ewe numbers have decreased by more than one-third or 10M during the last 10 years, and to increase stocking rates in higher rainfall zones. Barriers to more widespread utilisation of winter cereals for grazing have been the increased risks of metabolic disorders and ewe mortality, uncertainty about the benefits of mineral supplements and the absence of well-defined management guidelines for grazing pregnant and lactating ewes. To this end, MLA and AWI co-funded a 3 year project '*Fail Safe guides for grazing pregnant and lambing ewes on* cereals' (ON-00227) within the National Sheep Reproduction Strategy. During the first 12 months the project completed all milestones to the satisfaction of both funders, including: (*i*) literature review '*Grazing crops – implications for reproducing* sheep'; (*ii*) consultation with producers and consultants to identify the benefits and risks of grazing cereal crops with reproducing ewes; (*iii*) identification of the high priority areas for further research and development; and (*iv*) quantification of the potential economic benefit for the sheep industry from development of failsafe guidelines for grazing cereals.

About 30% of producers that currently graze cereal crops have experienced metabolic issues and increased mortality with pregnant ewes, and an additional 20% no longer graze crops with pregnant ewes as current recommendations have failed to provide a practical solution to eliminate these risks. This current project addresses the two highest priority areas for R&D identified from ON-00227. The first of these is to determine the mineral status of young crops and of the ewes grazing these cereal crops during the last third of pregnancy. The second priority is to test the effectiveness of mineral supplements to minimise the risk and incidence of metabolic disorders and reduce the mortality of pregnant ewes grazing cereal crops.

2 Project objectives

By 31st March 2017

1. Measured the changes in mineral status of ewes grazing winter crops on commercial farms during the last third of pregnancy
2. Determined if direct measures on mineral status in ewes can be used to predict the risk of metabolic disease
3. Determined the production responses of ewe grazing winter crops to mineral supplements in late pregnancy
4. Refined management strategies that must be followed to reduce the risk of ewe mortality and have these in a format to be road-tested by the national delivery networks such as Making More from Sheep and Bred Well Fed Well.

3 Mineral status of reproducing ewes grazing vegetative cereal crops (Experiment 1)

3.1 Background

Investigation into the macro-mineral composition of young growing wheat crops indicates a potential mineral imbalance. Potassium (K) concentration is 8-10 times higher than animal requirements and sodium (Na) is usually well below requirement, while calcium (Ca) and magnesium (Mg) usually meet requirements but are sometimes below. The high K, combined with low Na depresses Mg absorption and increases the risk of hypomagnesaemia. The low Ca in combination with a high dietary cation-anion difference (DCAD) increases the risk of induced Ca deficiency manifest as hypocalcaemia. In addition, a direct Na deficiency may be caused. Reproducing ewes already have an increased risk of hypocalcaemia and hypomagnesaemia and grazing young cereal crops will exacerbate this risk. Producers, particularly in eastern Australian, have reported higher ewe mortality and increased metabolic disease associated with the increasingly popular practice of grazing young cereal crops. To date there have been analyses of plants and reports of metabolic disease but few attempts to directly measure changes in grazing livestock. This experiment will directly monitor metabolic indicators in pregnant ewes grazing young vegetative crops. It is hypothesised that Mg and Ca status of these ewes will be low in some but not all grazing situations and that risk of metabolic disease is increased by grazing crops.

3.2 Objectives

1. Measure the changes in mineral status of ewes grazing young cereal crops during the last third of pregnancy.
2. Determine if direct measures on mineral status in ewes can be used to predict the risk of metabolic disease.

3.3 Methodology

Experiments were approved by Murdoch University's Animal Ethics Committee and all procedures were performed in accordance with the guidelines of the Australian Code of Practice for the Use of Animals for Scientific purposes.

3.3.1 Sites

Three research nodes were established prior to the initiation of this phase of the project. Within each node were individuals with both expertise and interest in the benefits and problems associated with grazing cereal crops with pregnant ewes. The leaders of each node were:

Dr Serina Hancock, Western Australia

Dr Susan Robertson, Wagga Wagga, NSW

Dr Gordon Refshauge, Cowra, NSW

Each node leader was responsible for farmer consultation, site establishment and protocol execution for their node. A central process of sample analysis was agreed, with all plant and soil samples were analysed by CSBP in Western Australia and all animal samples analysed through the Veterinary Diagnostic Laboratory and Environmental Analysis Laboratory at Charles Sturt University.

The experimental protocol was implemented on 18 farms. Six of these farms were located in the south west of Western Australia (primarily in the wheat-sheep zone) and a further 12 farms were located in southern NSW. Locations are shown on Figure 1 and sheep, crop types and experimental period are shown in Table 1.

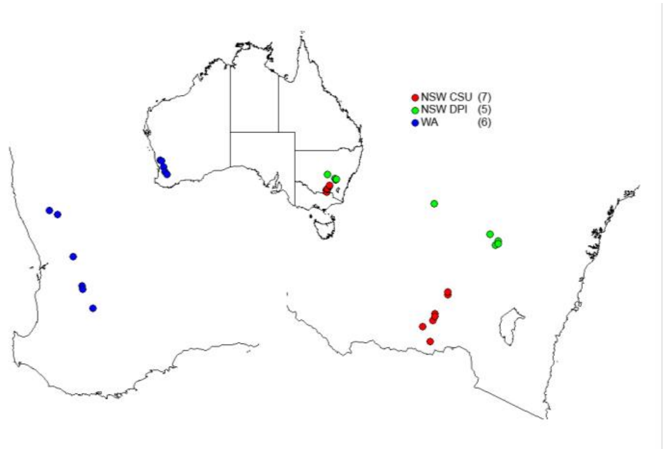

Fig 1. Location of experimental sites in WA (left frame), NSW (right frame), national (inset)

Table 1. Farms, location, sheep and crop information, and grazing times for the 17 experimental sites.

State	Location	Ewe number	Breed	Age (years)	Crop type	Pregnancy status	Grazing started	Gestational age start of grazing	Grazing ceased	Grazing days
WA	Northam	200	Merino	3 - 5	oats	twin	22/5/15	120	12/6/15	21
WA	Moora	220	Merino	2 - 5	barley	twin	9/6/15	127	30/6/15	21
WA	Pingelly	231	Merino	5	oats	twin	16/6/15	132	30/6/15	14
WA	Moora 2	176	Merino	2 - 4.5	barley	twin	23/6/15	133	8/7/15	15
WA	Pingelly	172	Merino	2 - 7	barley	twin	26/6/15	132	10/7/15	14
SNSW	Old Junee	270	Merino	5 + 7	oats (Bimble)	mainly twins	5/6/15	118	25/6/15	20
SNSW	The Rock	180	Merino	3 - 8	wheat (Wedgetail)	pregnant	11/6/15	117	1/7/15	20
SNSW	The Rock	175	Merino	3 - 8	wheat(Wedgetail)	pregnant	11/6/15	117	1/7/15	20
SNSW	Old Junee	104	Merino	4	oats (Bimble)	pregnant	12/6/15	118	3/7/15	21
SNSW	Burrumbuttock	200	Merino	3 - 4	wheat (Wedgetail)	single	29/6/15	122	20/7/15	21
SNSW	Yerong Creek	411	First X	4	wheat (Whistler)	pregnant	24/6/15	100	17/7/15	23
SNSW	Gerogery	449	Merino	6	wheat	pregnant	24/7/15	97	17/8/15	24
CNSW	Cowra	89	MM	3 - 6	Wheat (Wedgetail)	twin	12/6/15	120	3/7/15	21
CNSW	Cowra	91	MM	3 - 6	Oats (Yiddah)	twin	12/6/15	120	3/7/15	21
CNSW	Goologong	100	BLM	3-4	wheat (Gregory)	twin	22/6/15	112	13/7/15	21
CNSW	Trundle	150	BLM	2 - 6	Wheat (Wedgetail)	twin/singles	24/6/15	113	15/7/15	20
CNSW	Cowra	100	BLM	2 - 6	Barley (Erambie)	twin	16/7/15	113	6/8/15	22

3.3.2 Experimental protocol

On each of the 18 farms a mob of 150-400 pregnant ewes in late gestation (day 97-133 days after the start of mating) were placed in paddocks to graze cereal crops (wheat (8), barley (4) or oats (6)). Where possible twin bearing ewes were used. At the start of grazing, a total of 50 ewes were body condition scored from each mob and 15 of these ewes were randomly selected to have blood and urine samples collected. The mob grazed the crops for 2 to 3 weeks after which the same 50 ewes were condition scored, and blood and urine samples were collected from the same 15 ewes.

Prior to and post-grazing, two 9 ml blood samples were collected by jugular venipuncture into heparinised vacutainers and placed on ice before being centrifuged for 10 minutes. A 20 ml urine sample was collected via temporary nasal occulsion.

Forage samples were collected as grab samples from at last 10 equidistant sites located along a transect across the paddock. Collection was according to the instructions provided by the analytical laboratory (http://csbp-fertilisers.com.au/csbp-lab). At the time of forage collection, feed-on-offer (FOO) was also measured and soil samples were collected according to the instructions provided by the analytical laboratory (http://csbp-fertilisers.com.au/csbp-lab).

3.3.3 Sample processing and analysis

Forage samples were dried at 60^{o} C and soil samples were dried at 40^{o} C prior to delivery to a commercial analytical laboratory (CSBP Soil and Plant Analysis Laboratory, Bibra Lake, WA). Forage was analysed according to the CSBP standard analysis (nitrogen (N), phosphorus (P), K, sulfur (S), copper (Cu), zinc, (Zn), manganese (Mn), Ca, Mg, Na, iron (Fe), boron (B), nitrate and chloride) and soils according to the CSBP comprehensive analysis (Colwell P and K, sulfur (KCl 40), organic carbon, nitrate N, ammonia N, electrical conductivity, pH (water), pH ($CaCl_2$), B, trace elements [DTPA Cu, Zn, Mn and Fe] and exchangeable cations [Ca, Mg, Na, K and aluminium (Al)]).

Immediately after collection (on-site), specific gravity and pH of urine were measured (Pocket Refractometer, PAL-1, Atago, Japan in WA and CNSW and an FG302/312 portable refractometer, Australian Instrument Services, Melbourne used in SNSW). Sub-samples of plasma and urine were then placed on ice for transport before being frozen prior to being transported to the laboratory. Plasma and urine samples were analysed for concentrations of K, Na, Mg, Ca, P (Environmental and Analytical Laboratories, Charles Sturt University, Wagga Wagga NSW) using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)). Creatinine concentrations were also measured using the kinetic Jaffe method (Creatinine BLOSRx78 kit) with a Beckman Coulter AU480 analyser (Veterinary Diagnostic Laboratory, Charles Sturt University, Wagga Wagga, NSW). The concentrations of Ca, Mg and Na in urine were converted from mg/L to µmol/mosmol using the equation published by English and Hogan (1979); osmolality = -39231 + 39214*specific gravity.

3.3.4 Statistical analysis

Analysis of variance was used to determine the significance of differences in minerals in plants and soils between locations and crop types. A correlation matrix was used to identify relationships between forage and soil minerals (Statistica 10, StatSoft. Inc.). Multi and univariate regression analysis was used to identify the input factors (forage and soil composition, forage risk indices, crop type) most closely associated with the increased risk of hypocalcaemia or hypomagnesaemia (high urine pH, low Ca fractional excretion and low Mg fractional excretion; GenStat, VSN International)

3.4 Results

The oat crop on one property in Western Australia was observed to contain 20% non-crop plant material growing within the crop at the start of grazing; this had increased to 50% by the end of grazing. For this reason, the results from this property have been excluded from the analysis below. Other crops were estimated to have <5% non-crop plant material.

3.4.1 Ewe Performance

Feed on Offer (FOO) at the start of grazing ranged from 66 to 3342 kg DM/ha and at the end of grazing ranged from 112 to 2388 kg DM/ha (Table 2). On all but 4 properties FOO increased during grazing. At the start of grazing condition score of the ewes ranged from 2.4 to 3.8 and at the end of grazing ranged from 2.6 to 3.8. On all but 2 properties, condition score increased during grazing, even on the property where initial FOO was 66 kg/DM/ha (2.6 to 3.0; Table 2). Only two ewes were diagnosed with hypocalcaemia during the grazing period. This was followed by another 8 ewe deaths in the same mob of 172 ewes within 8 days after monitoring ceased. These deaths were in the flock of sheep identified as having the lowest risk of hypocalcaemia (Farm 5, Pingelly WA). Ten other ewe deaths were documented across all other sites. These included deaths in the total grazing group, not just the ewes that were monitored.

3.4.2 Crop Composition

Mineral concentration of the crop at the start and finish were averaged to provide a representative concentration. This single value was used for statistical analysis. There were significant differences between mineral composition of crop types and between farms ($P<0.05$). Crops grown on WA sites were higher in Na, Mg and Ca than crops grown on NSW sites. Barley contained higher concentrations of Na, Mg and Ca than oats and oats contained higher concentrations of these elements than wheat. However, with the unbalanced design of treatments across states, there is confounding of crop and location effects that mean the effects of crop and location cannot be separated. The number of farms with forage minerals outside accepted limits was also determined (Table 3). A high proportion of farms had forage Ca (11/17) and Na (12/17) below published requirements, and the same proportion (12/17) with K above the maximum tolerable level. Some farms also had forage Mg (3/17) and S (1/17) below requirements. Forage from all farms had a DCAD at a level that could increase the risk of hypocalcaemia, 12 had an elevated risk of grass tetany and 12 an abnormal K/Na+Mg ratio. All of the wheat crops had an elevated risk of grass tetany, an abnormal K/Na+Mg ratio and were deficient in Na.

3.4.3 Soil Composition

Soil analysis indicated significant differences ($P<0.05$) between sites. K in soils from WA sites was less than 25% of that at NSW sites and, inversely, Na in WA soils was 300% of that at the NSW sites (Fig 2). These soil differences were then strongly associated with the differences in crop composition with Colwell K in soil having a strong and significant ($P<0.05$) negative association with Na, Ca and Mg concentrations in crop forage (Table 4).

Table 2. Feed availability (kg DM/ha) and ewe condition score at the start and end of grazing.

State/Node	Location	Feed on offer –start (kg DM/ha)	Feed on offer – end (kg DM/ha)	Condition score - start	Condition score - end
WA	Northam	171	182	3.4	3.0
WA	Moora	66	112	2.6	3.0
WA	Pingelly	259	270	3.1	3.4
WA	Moora	194	515	2.4	2.6
WA	Pingelly	172	373	2.8	3.0
SNSW	Old Junee	1121	1266	3.0	3.1
SNSW	The Rock	787	698	2.9	3.0
SNSW	The Rock	919	1133	3.0	3.2
SNSW	Old Junee	1132	914	2.9	3.2
SNSW	Burrumbuttock	694	219	2.9	2.9
SNSW	Yerong Creek	472	620	2.6	2.8
SNSW	Gerogery	644	1493	2.6	2.7
CNSW	Cowra	1689	1982	3.0	3.2
CNSW	Cowra	3342	2388	3.0	3.1
CNSW	Goologong	811	2206	3.1	3.4
CNSW	Trundle	1602	1704	3.4	3.8
CNSW	Cowra	1037	2184	3.8	3.6

Table 3. Mean concentration (from start and end of grazing) of major minerals in forage from crops and risk of mineral imbalance.

Farm number	Location	Crop type	Ca in forage (% DM)	Mg in forage (% DM)	P in forage (% DM)	K in forage (% DM)	Na in forage (% DM)	S in forage (% DM)	Cl in forage (% DM)	DCAD in forage (meq/100g DM))	Tetany index in forage	K/(Na + Mg) ratio in forage	Total N in forage (% DM)
Required level			>0.4[1]	>0.09[1]	>0.2[1]	>0.5/<3[1]	>0.09[1]	>0.2[1]	>0.1[1]	<12[2]	<2.2[3]	<6[4]	~2.2
2	Moora	Barley	0.58	0.26	0.51	3.97	0.85	0.44	3.21	20.6	2.0	1.7	6.0
4	Moora	Barley	0.61	0.27	0.50	2.73	0.89	0.47	1.52	36.1	1.3	1.2	5.6
5	Pingelly	Barley	0.95	0.23	0.51	3.19	0.48	0.46	2.07	15.5	1.2	2.1	5.9
18	Cowra	Barley	0.40	0.14	0.55	4.29	0.04	0.38	1.92	33.9	3.6	8.7	5.1
1	Northam	Oats	0.49	0.24	0.63	2.55	0.90	0.44	1.22	42.8	1.5	1.1	4.9
3	Pingelly	Oats	0.53	0.22	0.44	3.35	0.58	0.43	1.54	41.1	1.9	2.0	4.9
7	Old Junee	Oats	0.34	0.09	0.21	2.55	0.02	0.20	0.38	42.8	2.7	7.9	2.5
10	Old Junee	Oats	0.26	0.09	0.24	2.58	0.01	0.19	0.49	40.6	3.2	8.7	2.3
15	Cowra	Oats	0.28	0.17	0.39	4.70	0.07	0.39	0.28	91.2	4.3	7.1	4.3
8	The Rock	Wheat	0.24	0.09	0.25	3.08	0.04	0.27	0.71	43.7	4.2	9.2	3.8
9	The Rock	Wheat	0.22	0.11	0.24	3.70	0.04	0.32	0.68	57.3	4.8	8.8	4.7
11	Burrumbuttock	Wheat	0.41	0.11	0.35	3.62	0.01	0.34	0.65	53.5	3.1	9.9	4.4
12	Yerong Creek	Wheat	0.36	0.13	0.50	3.72	0.02	0.37	0.67	53.9	3.4	8.7	5.2
13	Gerogery	Wheat	0.29	0.11	0.30	2.89	0.01	0.35	0.38	41.7	3.2	8.1	4.9
14	Cowra	Wheat	0.26	0.10	0.35	3.99	0.01	0.34	0.36	71.3	4.9	12.7	5.0
16	Goologong	Wheat	0.27	0.18	0.48	4.71	0.03	0.34	1.12	69.1	4.3	7.5	5.1
17	Trundle	Wheat	0.23	0.10	0.24	3.57	0.01	0.27	0.76	53.2	4.7	10.8	4.0
Number outside limits			11	3	0	12	12	1	0	17	12	12	0

[1] Derived from Freer *et al.* (2007) and/or National Research Council (2005).

[2] Estimated from Takagi and Block (1991c), DCAD = (Na/0.023 + K/0.039)-(Cl/0.0355 + S/0.016).

[3] Risk index for cattle (Kempt and ‘t Hart 1957).

[4] Risk index for K, Mg, Na imbalance (Dove *et al.* 2016).

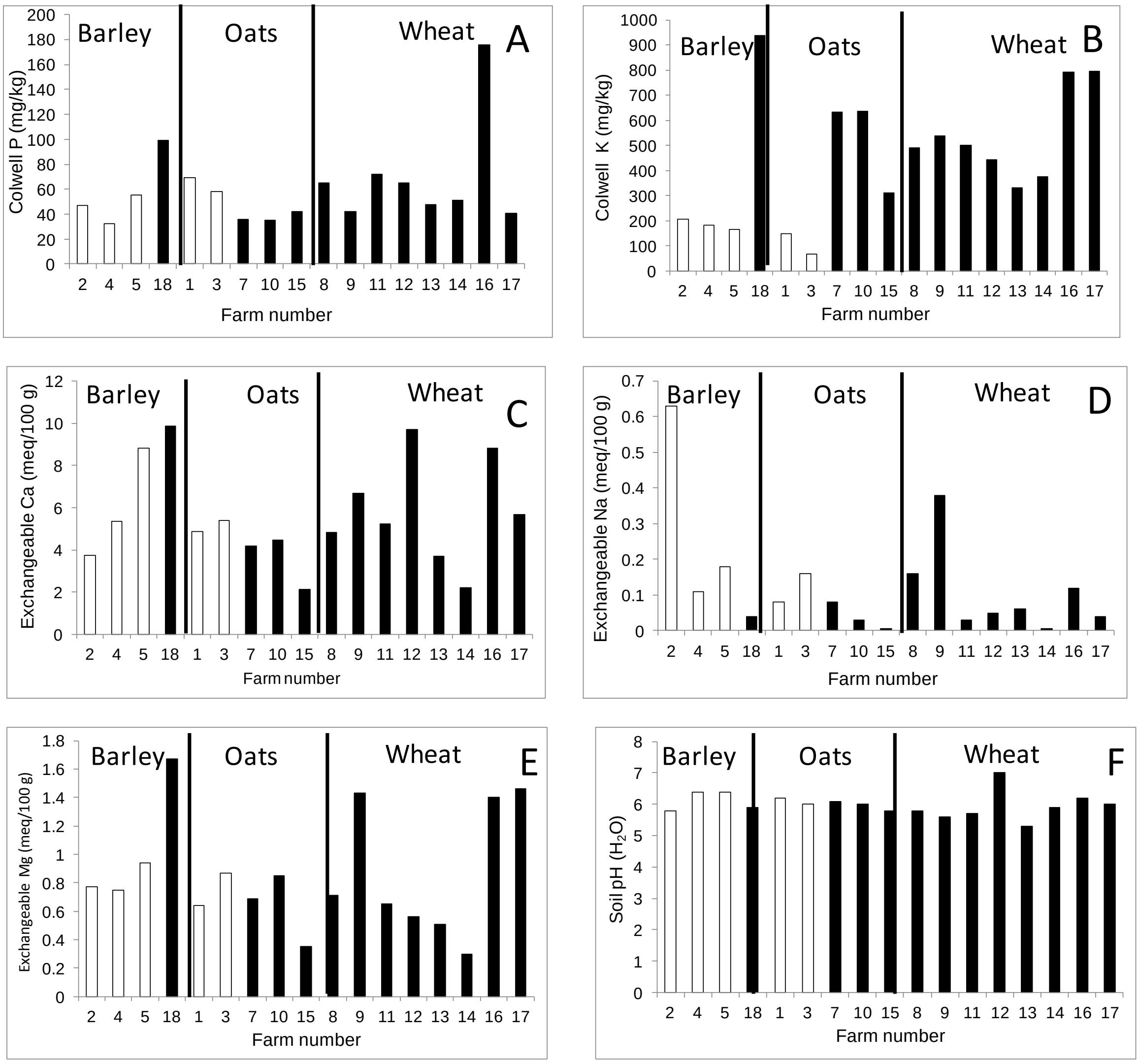

Fig 2. Soil Colwell P (A), Colwell K (B), exchangeable Ca (C), exchangeable Na (D), exchangeable Mg (E) and pH (F) at experimental farms growing barley, oats or wheat. Open bars are WA sites, black bars are NSW sites.

Table 4. Correlation matrix between mineral concentrations in soil and forage. Significant correlations shown in bold.

		Forage				Soil			
		Ca	Mg	Na	K	Exchangeable Ca	Exchangeable Mg	Exchangeable Na	Colwell K
Forage	Ca	1.00	**0.73**	**0.60**	-0.17				
	Mg	**0.73**	1.00	**0.85**	-0.05				
	Na	**0.60**	**0.85**	1.00	**-0.49**				
	K	-0.17	-0.05	**-0.49**	1.00				
Soil	Exchangeable Ca	0.25	0.04	-0.25	0.36	1.00	**0.68**	-0.13	0.45
	Exchangeable Mg	-0.05	-0.06	-0.24	0.35	**0.68**	1.00	0.08	**0.66**
	Exchangeable Na	0.26	0.44	**0.54**	-0.11	-0.13	0.08	1.00	-0.36
	Colwell K	**-0.55**	**-0.64**	**-0.75**	0.43	0.43	**0.66**	-0.33	1.00

3.4.4 Blood and urine analysis

There were significant differences among farms in urine pH, Ca, K, Mg and Na in plasma and fractional excretion of Ca, K, Mg and Na, but not P ($P<0.01$) (Tables 5 and 6). As with forage composition, the number of farms with measurements outside accepted limits was also determined. For some of the measurements, published limits were not available and this project will contribute to the establishment of these. Ewes on most farms had alkaline urine, high plasma K and low plasma Na, however there was no indication from plasma analysis that ewes were at risk of hypocalcaemia. Several studies have reported that fractional excretion of Ca and Mg can be used as an index to ascertain Mg and Ca status (see Bhanugopan *et al* 2015) and while there are no published guidelines, lower fractional excretion represents lower status for these minerals. Bhanugopan *et al* (2015) reported Ca fractional excretion of 0.47 – 0.57 % and Mg fractional excretion of 8.9 – 10.5 % in ewes fed diets with marginal levels of Ca and Mg and high K. Thirteen farms had Ca fractional excretion below this range and 9 farms had Mg fractional excretion below the range.

Table 5. Blood and urine metabolites in samples collected at the end of grazing.

Farm number	Location	Crop type	pH urine	Ca in plasma (mg/L)	K in plasma (mg/L)	Mg in plasma (mg/L)	Na in plasma (mg/L)	Ca in urine (µmol/mosmol)	Mg in urine (µmol/mosmol)	Na in urine (µmol/mosmol)
Required level			<7[1]	>90[2]	>94/<195[2]	>18[2]	>3220[2]	>1[3]	>1[4]	>1[5]
2	Moora	Barley	7.72	95.4 (3/14)[6]	202	22.6	2597	0.18	4.29	153.9
4	Moora	Barley	7.97	99.4 (2/11)	203	25.2	2615	0.33	4.48	57.4
5	Pingelly	Barley	6.75	102.7 (0/15)	225	21.9	2647	1.29	4.01	160.3
18	Cowra	Barley	8.23	104.5 (0/15)	182	24.1	2312	1.64	7.93	6.3
1	Northam	Oats	7.92	97.7 (3/17)	199	22.7	2618	0.85	6.04	128.6
3	Pingelly	Oats	7.73	104.4 (1/15)	217	26.3	2726	0.39	10.87	269.3
7	Old Junee	Oats	8.28	129.9 (0/15)	255	31.1	3078	0.07	1.40	122.5
10	Old Junee	Oats	8.24	117.8 (0/15)	235	30.3	2630	0.19	3.43	2.6
15	Cowra	Oats	7.82	108.1 (2/15)	206	26.1	2494	0.74	7.39	5.2
8	The Rock	Wheat	8.50	111.2 (0/14)	233	33.5	2782	0.07	2.41	4.2
9	The Rock	Wheat	8.52	112.0 (0/15)	235	31.7	3121	0.07	2.65	6.8
11	Burrumbuttock	Wheat	7.92	98.5 (2/14)	193	21.4	3091	0.10	0.36	1.0
12	Yerong Creek	Wheat	7.67	96.0 (4/13)	217	29.8	2380	0.10	3.24	0.6
13	Gerogery	Wheat	7.68	96.0 (4/13)	225	23.3	2470	0.08	1.05	2.9
14	Cowra	Wheat	8.12	109.6 (1/15)	222	25.3	2374	0.40	1.70	0.9
16	Goologong	Wheat	7.96	94.9 (4/15)	183	25.7	2206	0.30	4.39	5.1
17	Trundle	Wheat	8.16	109.0 (0/15)	209	27.7	2434	0.12	2.10	2.5
Number outside limits[7]			16	0	14	0	18	(15)[7]	1(3)[7]	3 (8)[7]

[1] Indicative only and should be used with other information (Grant *et al.* 1992).

[2] Values are for serum and are indicative only and should be used with other information (Suttle 2010).

[3] <1 µmol/mosmol indicates marginal status, no indicator for deficiency provided (Paynter 1996).

[4] Deficient range <1 µmol/mosmol, marginal 1-2 µmol/mosmol (Paynter 1996).

[5] Deficient range <1 µmol/mosmol, marginal 1-10 µmol/mosmol (Paynter 1996)

[6] Number in sampled group <90 mg/L, shown as number <90/total number sampled

[7] Number in parentheses indicate number of farms within the marginal range

Table 6. Fractional excretion (%) of Ca, K, Mg, Na and P in urine from samples collected at the end of grazing.

Farm number	Location	Crop type	Ca fractional excretion (%)	K fractional excretion (%)	Mg fractional excretion (%)	Na fractional excretion (%)	P fractional excretion (%)
2	Moora	Barley	0.23	122.2	13.7	4.36	0.14
4	Moora	Barley	0.32	93.5	10.9	1.70	0.25
5	Pingelly	Barley	1.63	114.1	14.4	5.01	0.31
18	Cowra	Barley	0.91	99.6	12.3	0.12	0.18
1	Northam	Oats	0.75	58.1	16.2	3.02	0.14
3	Pingelly	Oats	0.38	59.7	22.6	8.98	0.25
7	Old Junee	Oats	0.14	71.6	5.9	5.34	0.17
10	Old Junee	Oats	0.12	81.3	3.7	0.05	0.19
15	Cowra	Oats	0.65	128.9	11.3	0.13	0.24
8	The Rock	Wheat	0.06	58.1	4.1	0.11	0.16
9	The Rock	Wheat	0.05	49.3	4.1	0.09	0.13
11	Burrumbuttock	Wheat	0.14	113.0	1.0	0.03	0.17
12	Yerong Creek	Wheat	0.05	37.2	2.8	0.01	0.13
13	Gerogery	Wheat	0.10	97.7	3.1	0.08	0.31
14	Cowra	Wheat	0.36	157.1	3.4	0.03	0.29
16	Goologong	Wheat	0.33	88.0	10.2	0.17	0.38
17	Trundle	Wheat	0.18	100.4	5.1	0.20	0.22

3.4.5 Regression Analysis

The information on soil, forage, blood and urine all provide indicators of mineral supply and status, but on their own are an inefficient means of identifying risk and developing supplementation strategies. Regression analysis was used to identify the input factors (forage and soil composition, forage risk indices, crop type) most closely associated with the increased risk of hypocalcaemia or hypomagnesaemia (high urine pH, low Ca fractional excretion and low Mg fractional excretion).

Options for single-variate and multi-variate analysis were explored. With the data available, single variate regressions usually accounted for as much of the variation as multi-variate analysis.

Urine pH

Regression analysis for urine pH was run with forage Mg, forage Ca and Colwell K. Forage Ca accounted for the highest proportion of the variance (60%) and therefore had the strongest relationship with urine pH (Fig 3).

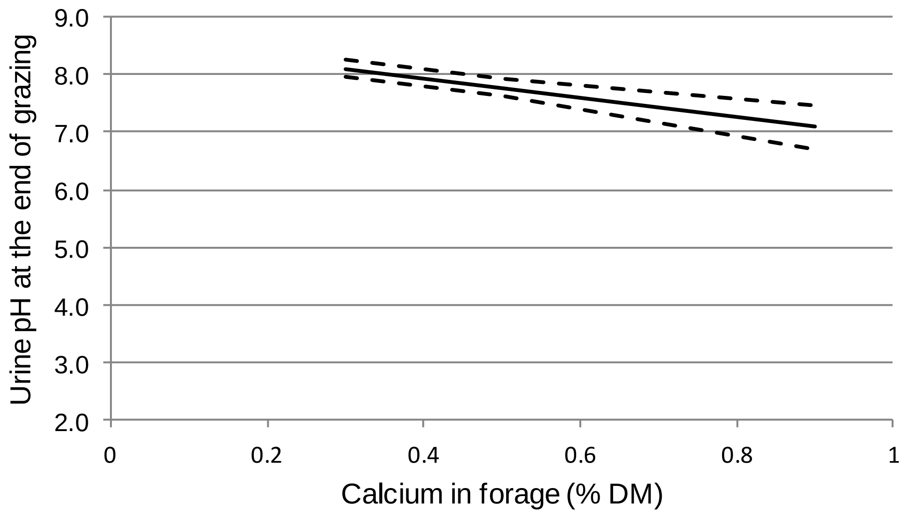

Fig 3. Relationship between Ca concentration in forage and urine pH at the end of grazing (x = 8.6 – 1.17y). Dotted lines represent 5% confidence intervals.

There was also a significant relationship between urine pH and both Mg in forage and Colwell K, but adding these to the regression equation did not improve prediction.

Magnesium fractional excretion

Regression analysis for urine Mg fractional excretion was run with forage Mg, forage Mg + crop type and the (K/Na+Mg) ratio in forage. Forage Mg alone accounted for 63% of the variance (Fig. 4) and when crop was added to the model these two factors combined accounted for 70 % of the variance. The K index when used alone accounted for 62% of the variance (Fig. 5).

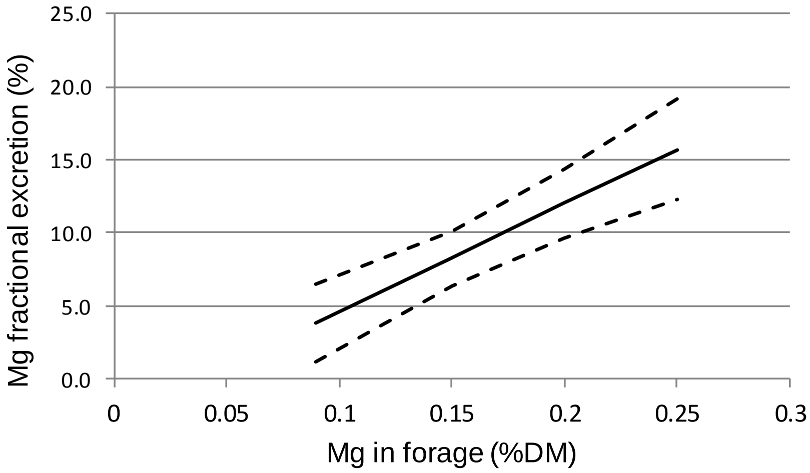

Fig 4. Relationship between Mg concentration in forage and Mg fractional excretion at the end of grazing (y = -2.89+74.4x). Dotted lines represent 5% confidence intervals.

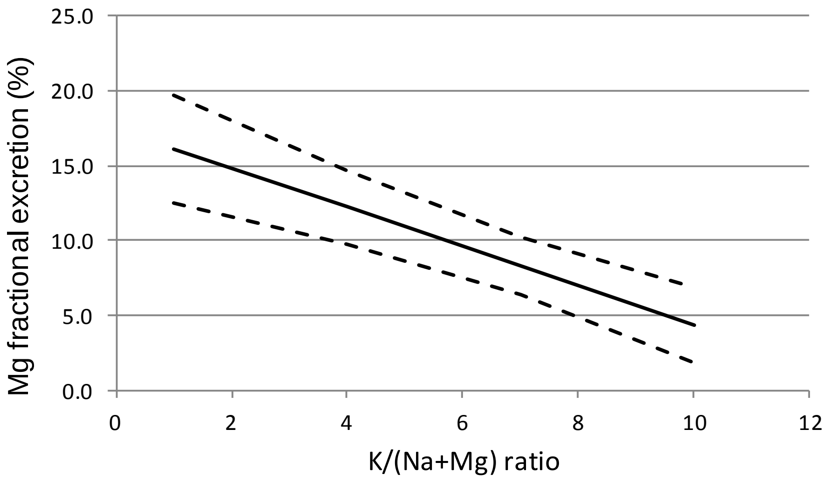

Fig 5. Relationship between the K index in forage and Mg fractional excretion at the end of grazing (y = 17.2 – 1.3x). Dotted lines represent 5% confidence intervals.

Calcium fractional excretion

Using the data available there were no input factors that were strongly related to Ca fractional excretion.

3.5 Discussion

The results support the hypothesis that the Ca, Mg and Na status of reproducing ewes grazing vegetative crops is low in some but not all grazing situations. The majority of the farms monitored had crop Ca concentrations below the published requirements for reproducing ewes of 0.4% DM (Freer *et al.* 2007). All crops measured had a high DCAD that was consistent with a decrease in the ewe's ability to mobilise Ca from bone during hypocalcaemia and a reduction in Ca retention (Takagi and Block 1991a, 1991c). A high risk of hypocalcaemia is usually accompanied by alkaline urine (Takagi and Block 1991a; Grant *et al.* 1992) and in individual sheep with plasma Ca below 90 mg/L (Suttle 2010). While none of the farm average plasma Ca concentrations were below 90 mg/L, at 10 of the 17 farms studied some ewes in the sampled flock had plasma Ca below 90 mg/L. The low to marginal Ca status of ewes was further reflected in low urine Ca concentrations, with mean urine Ca concentration on 15 of the 17 farms being below the upper limit for marginal Ca status (Paynter 1996). Several studies have reported that fractional excretion of Ca can be used as an index to ascertain Ca status (Bhanugopan *et al.* 2015) and while there are no published guidelines, lower fractional excretion represents lower status for these minerals. Bhanugopan *et al.* (2015) reported Ca fractional excretion of 0.47 – 0.57 % from ewes fed diets with marginal levels of Ca and Mg, and high K. Thirteen of the farms in this study had Ca fractional excretion below this range. Therefore, we conclude that reproducing ewes consuming vegetative crops are at risk of hypocalcaemia.

Farm 5 in Pingelly WA was the only property with ewes that had acidic urine and Ca concentrations in forage, plasma and urine well above deficiency levels. On this property DCAD was the lowest and fractional excretion of Ca the highest of all farms sampled, yet this property reported two cases of hypocalcaemia within the monitored flock. Stacey and Wilson (1970) suggest aciduria could have a direct effect of increasing urinary Ca excretion by preventing renal reabsorption of Ca. By increasing urinary Ca excretion, low DCAD diets trigger the homeostatic mechanism of bone mobilisation and increased intestinal absorption of Ca to maintain plasma Ca (Block 1984; Scott *et al.* 1993; Wilkens *et al.* 2016). Wilkens *et al.* (2012) showed that bone Ca mobilisation is an important mechanism for maintaining Ca homeostasis in sheep. When bone turnover is decreased for reasons such as age and increased physiological demands, it could result in breakdown of the homeostatic mechanism resulting in a decrease in plasma Ca and subsequently hypocalcaemia. It could be hypothesized that increased fractional excretion of calcium, with an acidic urine pH and breakdown of homeostasis could result in hypocalcaemia. Bone mobilisation of Ca is under the control of PTH and calcitriol. Therefore, elucidation of bone mobilisation markers and hormonal measurements of PTH and calcitriol could provide more insight into the mechanism. Other factors may also contribute. For example, susceptibility to hypocalcaemia is increased when ewes are fed green pastures following a period of low Ca grain feeding (Caple 1989). This may be a consequence of drought or seasonal dry periods (Larsen *et al.* 1986). Susceptibility to hypocalcaemia is also increased by a low vitamin D status, stress, or interrupted feeding, for example due to shearing, movement or inclement weather (Caple *et al.* 1988; Grant *et al.* 1988). The possible interaction between these factors and grazing vegetative crops requires further investigation. These risk factors however suggest that Ca supplements should be included in any grain rations fed to ewes over summer and autumn, and that handling of ewes consuming young cereal crops should be minimised.

In comparison to Ca concentrations, a smaller proportion of farms (17.6%) had forage Mg concentrations below published requirements of 0.09 %DM (Freer *et al.* 2007). However, the concentration of K was above the maximum tolerable limit of 3 %DM (National Research Council 2005) and the concentration of Na was below the required limit of 0.09 %DM on 12 of the 17 farms (Freer *et al.* 2007). As a consequence, the tetany index was above the risk level for cattle of 2.2 (Kempt and 't Hart 1957) and the K/(Na+Mg) index was above the normal range suggested for sheep of 6 (Dove *et al.* 2016). High K combined with low Na significantly decreases the absorption of Mg from the rumen and can induce a Mg deficiency in sheep consuming adequate Mg (Suttle and Field 1969; Greene *et al.* 1983). The imbalance of Mg with other minerals did not result in any of the flocks having a mean concentration of Mg in plasma in the deficient range of <18 mg/L (Suttle 2010). On only one farm was Mg in urine indicative of deficiency (<1 µmol/mosmol; (Paynter 1996). Fractional excretion of Mg has also been used as an index to ascertain Mg status where lower fractional excretion represents lower status. Bhanugopan *et al.* (2015) reported Mg fractional excretion of 8.9 to 10.5% in ewes fed diets with marginal levels of Ca and Mg, and high K. Fractional excretion was below this level on 9 farms in the current study. These observations are consistent with reports that hypomagnesaemia in pregnant ewes is rare (B Watt, pers comm.). Nevertheless, the marginal Mg status is likely to predispose ewes to hypocalcaemia (Herd 1965).

From a crop composition perspective, the results of this study indicate that, the risks of metabolic disease were highest for wheat, followed by oats, with the lowest risk in grazing barley. This is consistent with the conclusions of others (Dove *et al.* 2016), however the unbalanced design of this study means further research is required to support this conclusion. The plants were not grown on the same site. More interesting is the significant relationships between soil Colwell K and the mineral composition of all crops. There was a strong negative relationship between Colwell K and forage Ca, Mg and Na. The same relationship was not seen between forage K and forage Ca, Mg and Na. This indicates that Colwell K may influence the susceptibility to mineral imbalance. However the cause has not been established and further work is required, with a priority to determine if soil K directly influences other mineral in crops. Such information will be valuable to support decision making on supplement requirements for animals.

The composition of mineral supplements for ewes grazing crops is a further consideration. Traditionally a mixture of lime, causmag and salt (2:2:1) has been recommended (Dove and McMullen 2009; McGrath *et al.* 2015). The regression analysis indicates that low Ca in forage is associated with high urine pH and that low fractional excretion of Mg is associated with low forage Mg and Na, and high forage K. These results support the inclusion of Ca, Mg and Na in the supplement. However, some caution is required as the provision of Ca supplements to dairy cows can increased the risk of hypocalcaemia by resetting the homeostatic control of Ca to deal with excess Ca in the diet and not the sudden increase in mobilisation required at the time of milk letdown (McNeill *et al.* 2002). The relevance of high DCAD in the crop forage and in supplement formulation is uncertain. High DCAD is usually associated with a decrease in bone Ca accretion and high urine pH. However in this study, the association between DCAD and urine pH was weak ($r^2 = 0.12$, $P<0.1$). Others have reported a positive relationship between urine pH and DCAD (Takagi and Block 1991b; Grant *et al.* 1992). Provision of anionic salts to pregnant dairy goats and sheep has also resulted in reduced urine pH and elevated urine Ca (Liesegang 2008), although in the Liesegang (2008) study changes in urine pH and urine Ca were not associated with greater bone resorption. In these cited studies, DCAD treatments range from <-4 meq/100 g DM to >40 meq/100 g DM. The higher minimum DCAD (15.5 meq/100 g DM), together with the tight range (13 of the 17 farms between 30 and 70 meq/100 g DM) observed in crop forage may be the reason for the observed weak association in this study. On the basis of other published studies together with the measured DCAD in the current experiment, DCAD should not be ignored in supplement formulation. A lower DCAD would be preferable and this can partly be addressed by using chlorides and sulfates in the mineral mix rather than oxides or carbonates. This would suggest a supplement made from magnesium chloride, calcium sulphate and salt may be preferable to the traditional mixture.

While this study focussed on the mineral status of ewes grazing vegetative crops, other useful information was also obtained. Others have indicated that prior to grazing, crops should be well anchored, which occurs when FOO is around 1000 – 1500 kg DM/ha (Radcliffe *et al.* 2012). In the current study ewes grazed crops with FOO of <200 kg DM/ha and increased condition score, and this is more consistent with the observations of others (Virgona *et al.* 2006) (DR Thomas pers comm.). Delaying grazing of crops until they reach 1000 to 1500 kg DM/ha means there is an unnecessary risk of underutilisation of edible biomass, a loss of synchronisation between crop growth and the winter feed gap, and a reduction in the time available for grazing before the crop matures to reach jointing; stages Z30-Z31 (Zadoks *et al.* 1974), after which time further grazing will reduce grain yield (Harrison *et al.* 2011). On the condition that the crop roots are still well anchored, grazing at FOO of less than 500 kg DM/ha is a low risk option.

In summary, vegetative cereal crops can provide a high nutritive value forage suitable for grazing reproducing ewes, even when FOO is <500 kg/ha. However, the forage has a complex mineral composition that means the grazing ewe may have an increased risk of direct or induced Ca deficiency (hypocalcaemia) or Mg deficiency (hypomagnesaemia). The low Na and high K content of these crops may also pose a direct risk to production. Our analysis indicates the risks of metabolic disease are higher when grazing wheat and/or crops grown in soils with high K.

4 Mineral status of reproducing ewes grazing vegetative cereal crops with or without mineral supplementation (Experiment 2)

4.1 Background

Investigation into the macro-mineral composition of young growing wheat crops indicates a potential mineral imbalance. Potassium (K) concentration is 8-10 times higher than animal requirements and sodium (Na) is usually well below requirement, while calcium (Ca) and magnesium (Mg) are around requirements but sometimes below. The high K, combined with low Na depresses Mg absorption and increases the risk of hypomagnesaemia. The low Ca in combination with a high dietary cation-anion difference (DCAD) increases the risk on induced Ca deficiency manifest as hypocalcaemia. In addition, a direct Na deficiency may be caused. Reproducing ewes already have an increased risk of hypocalcaemia and hypomagnesaemia and grazing young wheat crops will exacerbate this risk. Producers, particularly in eastern Australian, have reported higher ewe mortality and increased metabolic disease associated with the increasingly popular practice of grazing young cereal crops.

In the first year of this project (phase 1), a review of the literature, combined with survey and interview of producers and analysis of crop plants confirmed the potential for mineral imbalance and metabolic disease. During investigation of the literature it was apparent that there had been few attempts to directly measure changes in grazing livestock. This was addressed in the second year (phase 2, experiment 1). Preliminary investigation of results from phase 2 has indicated the risks of metabolic disorders (as defined by reduced urine pH and low fractional excretion of Ca and Mg) were positively related to soil exchangeable K (high soil K, high risk) and negatively related to forage Ca, Mg, Na and to a lesser extent low DCAD. These results provide the basis for the formulation, feeding and evaluation of a supplement for ewes grazing crops in late pregnancy. The hypothesis for this study is in two parts. The first is that a mineral supplement providing Ca, Mg and Na to pregnant ewes grazing vegetative crops will improve Ca and Mg status of the ewes and, the second is that a mineral supplement designed to reduce DCAD while providing Ca, Mg and Na, will be more effective than a supplement that has no influence on DCAD.

4.2 Objectives

Measure the response to mineral supplements in pregnant ewes grazing young cereal crops during the last third of pregnancy.

4.3 Methodology

Experiments were approved by Murdoch University's Animal Ethics Committee for DPI and WA sites and by Charles Sturt University for the CSU sites. All procedures were performed in accordance with the guidelines of the Australian Code of Practice for the Use of Animals for Scientific purposes.

4.3.1 Sites

As with experiment 1, three research nodes were established. Within each node were individuals with both expertise and interest in the benefits and problems associated with grazing cereal crops with pregnant ewes. The leaders of each node were:

Dr Serina Hancock, Western Australia

Dr Susan Robertson, Wagga Wagga, NSW

Dr Gordon Refshauge, Cowra, NSW

Each node leader was responsible for farmer consultation, site establishment and protocol execution for their node. A central process of sample analysis was agreed, with all plant and soil samples analysed by CSBP in Western Australia and all animal samples analysed through the Veterinary Diagnostic Laboratory and Environmental Analysis Laboratory at Charles Sturt University.

The experimental protocol was implemented on 7 farms across Western Australia (3 farms) and the southern and central regions of NSW (4 farms) (Fig 6). Where possible the farms were a subset of those monitored in experiment 1 and were selected for high metabolic disease risk based on high urine pH, high soil exchangeable K, low forage Mg, Ca and Na (Table 7) and/or the incidence of metabolic disease in experiment 1.

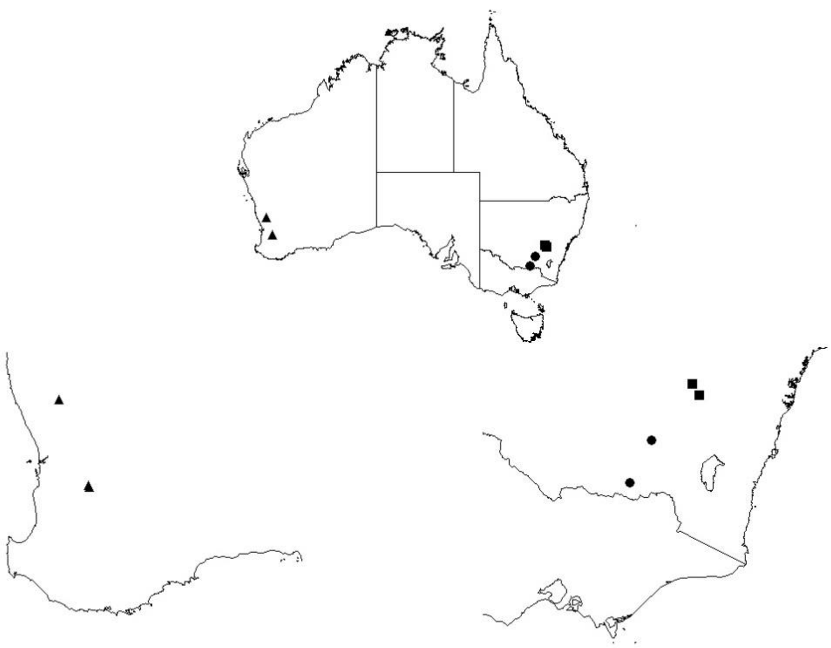

Fig 6. Location of sites for experiment 2 in WA (left frame), NSW (right frame), national (inset)

Table 7. Matrix for site selection.

Urine pH	Ca fractional excretion	Mg fractional excretion	Soil Colwell K	Forage Na	Incidence of metabolic disease
>7.9	<0.25	<5.5	>410	<0.035	Y/N

4.3.2 Experimental protocol

On each of the seven farms a total of 90 twin bearing ewes in late gestation (d115 to d129 of gestation) were divided into three treatment groups (n=30/treatment). One group of ewes were a control group and given no supplement. A second group was provided *ad libitum* (up to 30 g/day) access to the industry standard (causmag, limestone, salt, 2:2:1). The third group was provided with *ad libitum* (up to 30 g/day) access to a supplement specifically designed to address the metabolic imbalance as shown below in Table 8.

Table 8. Proposed composition of experimental mineral mix.

Mineral	Component	Percent of mix
Magnesium	$MgCl_2.6H_2O$	12.5
Calcium	$CaSO_4.2H_2O$	32.5
Salt	NaCl	55.0

DCAD ~ -550

The ewes were placed in paddocks to graze cereal crops (wheat (4), barley (1) or oats (2)) for a total of three weeks with the supplement offered to the supplemented groups for the entire duration. The supplements were provided in troughs with a small roof to minimise rain entering the troughs. At the end of each week, each flock was rotated to the adjoining plot, so, by the end of the three-week grazing period each group of ewes grazed each of the three plots. Supplements were moved with the ewes and any residue around the troughs was also removed.

At the start of grazing, all ewes were body condition scored and 10 of these ewes were randomly selected to have blood and urine samples collected. At the end of three weeks all the ewes were condition scored again and blood and urine was collected from the same 10 ewes. On four farms, the ewes were Merino, on two they were Border Leicester/Merino cross and on the other farm the ewes were White Suffolk/Merino cross (Table 9).

Two 9 ml blood samples were collected by jugular venipuncture into heparinised vacutainers and placed on ice before being centrifuged 10 minutes. A 20 ml urine sample was collected via temporary nasal occulsion.

Forage samples were collected as grab samples from at least 10 equidistant sites located along a transect across the paddock. Collection was according to the instructions provided by the analytical laboratory (http://csbp-fertilisers.com.au/csbp-lab). At the time of forage collection FOO was also measured and soil samples were also collected according to the instructions provided by the analytical laboratory (http://csbp-fertilisers.com.au/csbp-lab).

4.3.3 Sample processing and analysis

Forage samples were dried at 60^o C and soil samples were dried at 40^o C prior to delivery to a commercial analytical laboratory (CSBP Soil and Plant Analysis Laboratory, Bibra Lake, WA). Forage was analysed according to the CSBP standard analysis (nitrogen (N), phosphorus (P), K, sulfur (S), copper (Cu), zinc, (Zn), manganese (Mn), Ca, Mg, Na, iron (Fe), boron (B), nitrate and chloride) and soils according to the CSBP comprehensive analysis (Colwell P and K, sulfur (KCl 40), organic carbon, nitrate N, ammonia N, electrical conductivity, pH (water), pH ($CaCl_2$), B, trace elements [DTPA Cu, Zn, Mn and Fe] and exchangeable cations [Ca, Mg, Na, K and aluminium (Al)]).

Immediately after collection (on-site), specific gravity and pH of urine was measured (Pocket Refractometer, PAL-1, Atago, Japan in WA and CNSW and an FG302/312 portable refractometer, Australian Instrument Services, Melbourne used in SNSW). Sub-samples of plasma and urine were then placed on ice for transport before being frozen prior to being transported to the laboratory. Plasma and urine samples were analysed for concentrations of K, Na, Mg, Ca, P (Environmental and Analytical Laboratories, Charles Sturt University, Wagga Wagga NSW) using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Creatinine concentrations were also measured by the kinetic Jaffe method (Creatinine BLOSRx78 kit), using a Beckman Coulter AU480 analyser (Veterinary Diagnostic Laboratory, Charles Sturt University, Wagga Wagga, NSW). The concentrations of Ca, Mg and Na in urine were converted from mg/L to µmol/mosmol using the equation published by English and Hogan (1979); osmolality = -39231 + 39214*specific gravity.

4.3.4 Statistical analysis

Analysis of variance was used to determine the significance of changes related to farm and treatment. Where the interaction was significant main effects of farm or treatment were not analysed independently. When either farm x treatment or treatment effects were significant, the significance of the differences between the three treatment within each individual farm were determined using Least Significant Difference (Statistica 10, StatSoft. Inc., Tulsa, OK, 74104, USA).

Table 9. Farms, location, sheep and crop information and grazing times for the six experimental sites

State	Location	Treatment[a]	Ewe numbers	Breed[b]	Ewe age (years)	Crop type	Pregnancy status	Grazing started	Grazing ceased	Grazing days	Gestational age
WA	Pingelly	1	30	Merino	3-5	Oats	Twins	31/5/16	21/6/16	21	115
WA	Pingelly	2	30	Merino	3-5	Oats	Twins	31/5/16	21/6/16	21	115
WA	Pingelly	3	30	Merino	3-5	Oats	Twins	31/5/16	21/6/16	21	115
WA	Moora	1	30	Merino	3-5	Barley	Twins	2/6/16	23/6/16	21	117
WA	Moora	2	30	Merino	3-5	Barley	Twins	2/6/16	23/6/16	21	117
WA	Moora	3	30	Merino	3-5	Barley	Twins	2/6/16	23/6/16	21	117
WA	Pingelly	1	30	Merino	3-5	Barley	Twins	22/6/16	13/7/16	21	129
WA	Pingelly	2	30	Merino	3-5	Barley	Twins	22/6/16	13/7/16	21	129
WA	Pingelly	3	30	Merino	3-5	Barley	Twins	22/6/16	13/7/16	21	129
NSW	Cowra	1	30	Merino	3-7	Wheat	Twins	17/6/16	8/7/16	21	122
NSW	Cowra	2	30	Merino	3-7	Wheat	Twins	17/6/16	8/7/16	21	122
NSW	Cowra	3	30	Merino	3-7	Wheat	Twins	17/6/16	8/7/16	21	122
NSW	Gooloogong	1	30	BLM	3-7	Wheat	Twins	4/7/16	25/7/16	21	128
NSW	Gooloogong	2	30	BLM	3-7	Wheat	Twins	4/7/16	25/7/16	21	128
NSW	Gooloogong	3	30	BLM	3-7	Wheat	Twins	4/7/16	25/7/16	21	128
NSW	Walla	1	30	BLM	3-7	Wheat (wedgetail)	Twins	1/7/16	22/7/16	21	108
NSW	Walla	2	30	BLM	3-7	Wheat (wedgetail)	Twins	1/7/16	22/7/16	21	108
NSW	Walla	3	30	BLM	3-7	Wheat (wedgetail)	Twins	1/7/16	22/7/16	21	108
NSW	Junee	1	30	WSM	3+	Wheat (wedgetail)	40%twins	21/6/16	12/7/16	21	121
NSW	Junee	2	30	WSM	3+	Wheat (wedgetail)	40%twins	21/6/16	12/7/16	21	121
NSW	Junee	3	30	WSM	3+	Wheat (wedgetail)	40%twins	21/6/16	12/7/16	21	121

[a] Treatment 1, no minerals, treatment 2 industry standard (causmag, limestone, salt), treatment 3 new supplement (magnesium chloride, gypsum, salt). [b]Breed – Merino, Border Leicester x Merina (BLM) or White Suffolk x Merino (WSM).

4.4 Results

The oat crop on one property in Western Australia had sheep mixing in between plots and hence was excluded from the analysis.

4.4.1 Ewe Performance

There was one ewe death during the experimental period. This ewe was in the New supplement group and farm 1 (Pingelly, WA). The cause of death was unknown.

Condition score of ewes at the start of grazing ranged from 2.7 to 3.5 and at the end of grazing ranged from 2.7 to 3.8. In all but one of the groups, condition score increased during grazing. There were significant differences among farms but treatment did not have any significant effect on condition score at the end of grazing or on condition score change during grazing ($P>0.05$) (Table 10).

FOO at the start of grazing ranged from 221 to 1877 kg DM/ha and at the end of grazing ranged from 112 to 2388 kg DM/ha. On all properties, FOO increased during grazing.

4.4.2 Minerals in crops and soils

Mineral concentration in the crop at the start and finish were averaged to provide a representative concentration over the grazing period. This single value was used for comparisons between the supply of minerals from forage and the published requirements. All of the four farms with wheat as forage were from NSW and all of these had concentrations of Ca and Na below published requirements and DCAD, grass tetany index and K/(NA+K) ratios that were indicative of increased risk of induced Mg deficiency. Forages on all six experimental farms had forage K above the maximum tolerable level and DCAD indicative of increased risk of Ca deficiency (Table 11). Soil analysis indicated high variation in Colwell K, P, and exchangeable Ca, Mg and Na across location (Table 12).

4.4.3 Mineral supplement consumption

Ewes on all farms consumed at least 70% of the New supplement offered. Consumption of Standard supplement was more variable ranging from 4.9 to 30 g/day. There was no statistically significant difference in intake between the two supplements ($P=0.15$). For farm 3 (West Pingelly, WA) a small amount of lupins (approximately 40 g/sheep/day) was sprinkled on top to the supplements to attract the ewes. The same amount of supplement was feed to all ewes in all treatment groups on this farm.

Table 10. Feed on offer (FOO; kg DM/ha) and ewe condition score at the start and end of grazing.

Farm no	Location	State	Crop type	Treat	FOO On (kg/DM/ha)	FOO Off (kg/DM/ha)	CS On	CS Off	CS change
1	Pingelly	WA	Oats	1	479	1488	3.2	3.3	0.09
1	Pingelly	WA	Oats	2	479	1488	3.3	3.4	0.07
1	Pingelly	WA	Oats	3	479	1488	3.2	3.3	0.08
3	West Pingelly	WA	Barley	1	191	348	3.3	3.7	0.48
3	West Pingelly	WA	Barley	2	191	348	3.5	3.7	0.25
3	West Pingelly	WA	Barley	3	191	348	3.4	3.8	0.38
4	Cowra	NSW	Wheat	1	1877	2287	2.7	2.7	-0.02
4	Cowra	NSW	Wheat	2	1877	2287	2.8	2.8	0.05
4	Cowra	NSW	Wheat	3	1877	2287	2.8	2.8	0.02
5	Gooloogong	NSW	Wheat	1	937	2339	2.7	2.9	0.15
5	Gooloogong	NSW	Wheat	2	937	2339	2.7	2.9	0.16
5	Gooloogong	NSW	Wheat	3	937	2339	2.7	2.9	0.21
6	Walla	NSW	Wheat	1	665	839	2.7	2.9	0.18
6	Walla	NSW	Wheat	2	665	839	2.7	2.9	0.20
6	Walla	NSW	Wheat	3	665	839	2.7	2.9	0.19
7	Junee	NSW	Wheat	1	221	428	3.0	3.3	0.26
7	Junee	NSW	Wheat	3	221	428	3.1	3.2	0.13
7	Junee	NSW	Wheat	2	221	428	3.0	3.3	0.25

Table 11. Mean concentration (from start and end of grazing) of major minerals in forage from crops and risk of mineral imbalance

Farm no	Location	Crop type	Ca in forage (% DM)	Mg in forage (% DM)	P in forage (% DM)	K in forage (% DM)	Na in forage (% DM)	S in forage (% DM)	Cl in forage (% DM)	DCAD in forage (meq/100g DM))	Tetany index in forage	K/(Na + Mg) ratio in forage	Total N in forage (% DM)
Required level			>0.4[1]	>0.09[1]	>0.2[1]	>0.5/<3[1]	>0.09[1]	>0.2[1]	>0.1[1]	<12[2]	<2.2[3]	<6[4]	~2.2
1	Pingelly	Oats	0.50	0.22	0.40	3.09	0.73	0.51	1.32	41.7	1.8	1.6	5.2
3	West Pingelly	Oats	1.12	0.24	0.44	3.25	0.36	0.48	1.37	30.1	1.1	2.4	5.3
4	Cowra	Wheat	0.31	0.14	0.48	5.05	0.01	0.36	0.36	97.4	4.9	11.2	5.1
5	Gooloogong	Wheat	0.24	0.13	0.53	4.00	0.02	0.36	0.38	70.5	4.5	8.9	4.5
6	Walla	Wheat	0.35	0.12	0.43	3.82	0.02	0.36	0.35	66.1	3.7	9.7	5.0
7	Junee	Wheat	0.28	0.15	0.54	4.53	0.02	0.44	0.40	77.9	4.4	8.9	6.0
Number outside limits			4	0	0	6	4	0	0	6	4	4	0

[1] Derived from Freer *et al.* (2007) and/or National Research Council (2005).

[2] Estimated from Takagi and Block (1991c), DCAD = (Na/0.023 + K/0.039)-(Cl/0.0355 + S/0.016).

[3] Risk index for cattle (Kempt and 't Hart 1957).

[4] Risk index for K, Mg, Na imbalance (Dove *et al.* 2016)

Table 12. Soil mineral concentrations

Farm no	Location	Exchangeable Ca (meq/100g)	Exchangeable Mg (meq/100g)	Colwell K (mg/kg)	Exchangeable Na (meq/100g)	Colwell P (mg/kg)
1	Pingelly	5.3	0.62	110	0.16	40
3	West Pingelly	8.9	1.00	206	0.18	54
4	Cowra	3.5	0.60	285	0.05	43
5	Gooloogong	3.7	0.52	479	0.03	55
6	Walla	4.0	0.39	305	0.02	62
7	Junee	2.5	0.54	421	0.02	40

4.4.4 Blood and urine analysis

There were significant differences related to farm, treatment or farm x treatment on urine pH and Ca, K, Mg and Na in plasma and urine and fractional excretion of Ca, K, Mg and Na but not P ($P<0.01$) (Table 13, Fig. 7). When the influence of treatments within farms was assessed, either one or both of the mineral supplements (New and Standard), significantly increased Ca in plasma and urine on five of the six farms and increased Ca fractional excretion on four of the six farms ($P<0.05$). Only the ewes on farm 7 (Junee, NSW) showed no Ca response to supplements (Table 13, Fig. 7). Ewes provided with either the New or Standard mineral supplement had significantly different concentration of Mg in plasma when compared to Control ewes on three of the six farms, only on two of these was the change an increase ($P<0.05$), on four farms ewes provided with one or other supplement had significantly higher Mg in urine ($P<0.05$; Table 12). There was no treatment effect of mineral supplementation on Mg fractional excretion (Fig. 7). Ewes provided with either the New or Standard mineral supplement had significantly different concentration of K in plasma and fractional excretion when compared to Control ewes on some farms ($P<0.05$) but changes were inconsistent among treatments (Table 13, Fig. 7). Similarly, there were inconsistent responses to supplements in urine Na and no influence of treatment on Na fractional excretion (Table 13, Fig. 7).

Table 13. Blood and urine metabolites in samples collected at the end of grazing.

Farm number	Location	Treatment	Crop type	Ca in plasma (mg/L)	Mg in plasma (mg/L)	K in plasma (mg/L)	Na in plasma (mg/L)	Ca in urine (µmol/mosmol)	Mg in urine (µmol/mosmol)	Na in urine (µmol/mosmol)
Required level				>90[1]	>18[1]	>94/<195[1]	>3220[1]	>1[2]	>1[3]	>1[4]
1	Pingelly	1	Oats	96.1[a5]	16.9[a5]	181[a5]	3275	0.84[a5]	4.1[a5]	173.7[a5]
1	Pingelly	2	Oats	116.3[b]	26.3[b]	231[b]	3143	2.22[b]	6.5[ab]	182.5[a]
1	Pingelly	3	Oats	117.0[b]	26.2[b]	215[b]	3075	1.44[ab]	8.7[b]	132.7[a]
3	West Pingelly	1	Barley	95.4[a]	21.2[a]	195[a]	2772	0.33[a]	5.0[a]	235.9[a]
3	West Pingelly	2	Barley	110.2[b]	24.5[a]	188[a]	3064	2.07[b]	10.3[ab]	704.5[b]
3	West Pingelly	3	Barley	106.4[b]	22.2[a]	185[a]	3149	1.98[b]	11.2[b]	494.3[ab]
4	Cowra	1	Wheat	90.4[a]	21.2[a]	194[a]	2823	0.46[a]	1.1[a]	59.9[a]
4	Cowra	2	Wheat	103.0[b]	20.5[a]	190[a]	2751	0.69[a]	3.4[a]	26.2[ab]
4	Cowra	3	Wheat	103.0[b]	24.4[b]	219[b]	2756	4.65[b]	5.1[a]	6.4[b]
5	Gooloogong	1	Wheat	99.8[a]	23.3[a]	243[a]	2892	0.34[a]	1.2[a]	2.3[a]
5	Gooloogong	2	Wheat	109.1[b]	18.5[b]	208[b]	2850	15.37[b]	32.2[b]	63.2[a]
5	Gooloogong	3	Wheat	107.6[ab]	20.2[b]	222[b]	2957	6.58[ab]	10.0[ab]	172.2[b]
6	Walla	1	Wheat	104.1[a]	26.2[a]	198[a]	2950	0.16[a]	2.5[a]	0.7[a]
6	Walla	2	Wheat	113.1[b]	24.1[a]	192[a]	2988	0.34[a]	3.0[a]	18.2[a]
6	Walla	3	Wheat	110.0[ab]	25.3[a]	214[a]	3171	1.18[b]	5.6[b]	21.9[a]
7	Junee	1	Wheat	113.0[a]	24.0[a]	207[a]	3087	0.82[a]	3.8[a]	17.5[a]
7	Junee	2	Wheat	117.5[a]	23.8[a]	209[a]	3027	0.67[a]	4.1[a]	24.1[a]
7	Junee	3	Wheat	109.1[a]	23.5[a]	214[a]	3020	0.58[a]	3.9[a]	20.3[a]
Statistical analysis				Farm*** Treatment***	Farm x Treatment	Farm x Treatment	Farm	Farm x Treatment	Farm x Treatment	Farm x Treatment

[1] Values are for serum and are indicative only and should be used with other information (Suttle 2010).

[2] <1 µmol/mosmol indicates marginal status, no indicator for deficiency provided (Paynter 1996).

[3] Deficient range <1 µmol/mosmol, marginal 1-2 µmol/mosmol (Paynter 1996).

[4] Deficient range <1 µmol/mosmol, marginal 1-10 µmol/mosmol (Paynter 1996).

[5] Different superscripts indicate significant difference (P<0.05), comparisons of treatments within farm only.

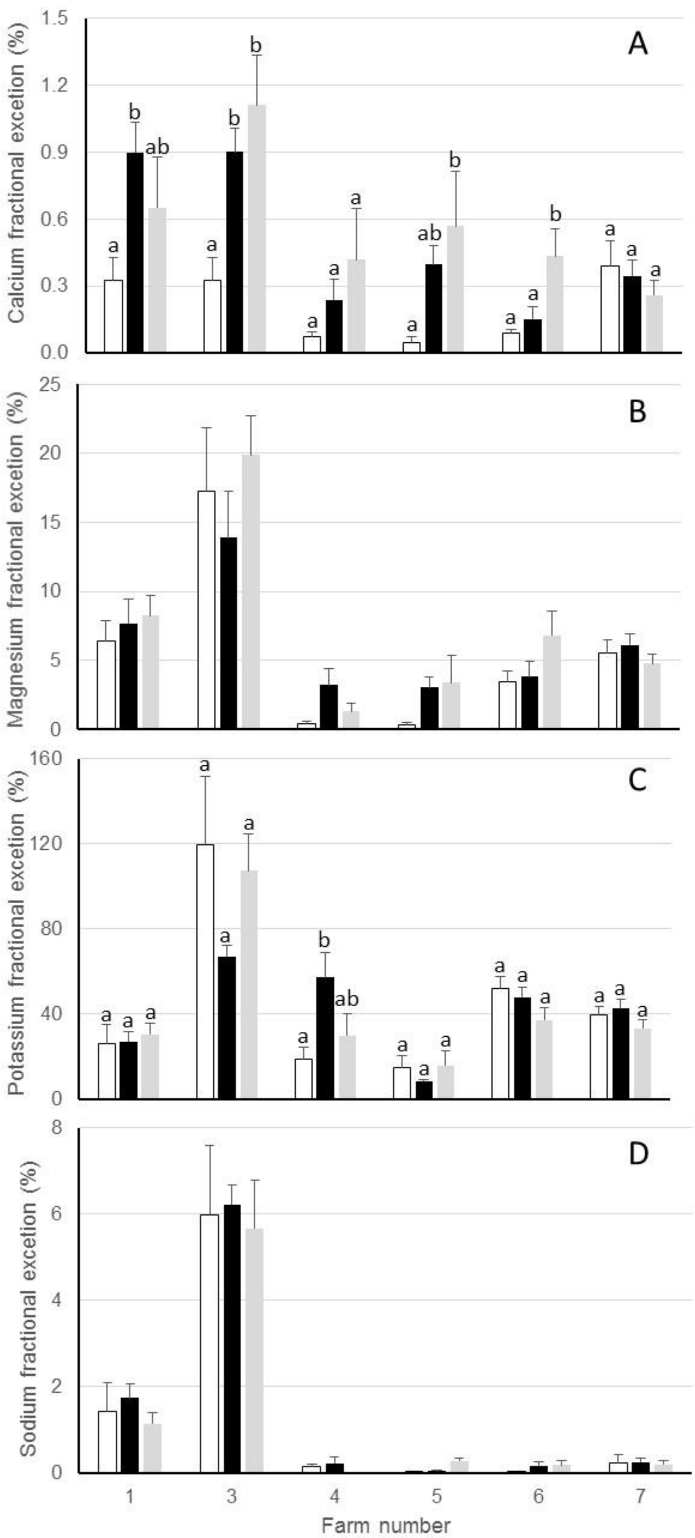

Fig 7. Mean fractional excretion of Ca (A), Mg (B), K (C) and Na (D) on each of seven farms from sheep fed no supplement (open bars), the Industry supplement (black bars) and the New supplement (grey bars). Within farms, bars with different letters are significantly different (P<0.05).

4.5 Discussion

The results support the hypothesis that Ca supplements provided to pregnant ewes grazing vegetative crops will improve Ca and Mg status and may decrease the risk of hypocalcaemia and hypomagnesaemia. On all but one of the six farms, ewes provided Ca supplements showed an increase in Ca concentration in urine and plasma. Mean concentrations of Ca in plasma in unsupplemented, Control ewes was above the marginal level on all farms, but on five farms, individual sheep within the Control groups were below adequate (<90 mg/L). Mean Ca in urine of Control ewes was below the adequate range (<1 µmol/mosmol) on all farms (Paynter 1996; Suttle 2010). These observations were similar to those previously reported in unsupplemented ewes grazing vegetative wheat, oats or barley (Masters *et al.* 2017). On some farms supplementation increased Ca concentrations above the marginal range whereas on others increases were significant but Ca status of the supplemented ewes was still marginal. Several studies have reported that fractional excretion of Ca can be used as an index to ascertain Ca status (Bhanugopan *et al.* 2015) and while there are no published guidelines, lower fractional excretion represents lower status for these minerals. Bhanugopan *et al* (2015) reported Ca fractional excretion of 0.47 – 0.57 % from ewes fed diets with marginal levels of Ca and Mg, and high K. Others have reported Ca fractional excretion between 0.05 and 1.63% in flocks of ewes grazing cereals (Masters *et al.* 2017). In the current study, mean fractional excretion of Ca in all Control groups was below 0.4% and either one or both of the Ca supplements increased fractional excretion of Ca above 0.4%. Hypocalcaemia is also usually accompanied by alkaline urine (Takagi and Block 1991a; Grant *et al.* 1992). Most flocks in this study had a urine pH above 7 and there was a significant farm x treatment effect on pH change (start minus end of grazing), with the New or Standard supplements resulting in a significant reduction in urine pH on three of the six farms.

Improvement in Mg status was less clear. Supplements only increased Mg in plasma on two farms and Mg in urine on four farms. There was no significant effect of treatment on Mg fractional excretion. Mean concentrations of Mg in plasma were slightly below or slightly above the concentration indicative of low status (<18 mg/L). Mg in urine indicated some groups of ewes were within the marginal range (1- 2 µmol/mosmol), but most had sufficient Mg (>2 µmol/mosmol) (Paynter 1996; Suttle 2010). Bhanugopan *et al.* (2015) reported Mg fractional excretion of 8.9 to 10.5% in ewes fed diets with marginal levels of Ca and Mg, and high K. Fractional excretion was below this level on five farms, even in supplemented ewes, indicating that critical fractional excretion values remain to be established.

The mineral status of ewes was a reflection of the mineral composition of the crops. All wheat forages had Ca concentrations below the published requirements for reproducing ewes (Ca <0.4%) (Freer *et al.* 2007) and DCAD above 60. DCAD above 12 has been associated with a decrease in the ewe's ability to mobilise Ca from bone during hypocalcaemia and a reduction in Ca retention (Takagi and Block 1991c, 1991a). Although the concentrations of Mg in forage were not below published requirements for any crop, Na concentrations in wheat forage were below the published requirements for reproducing ewes (Na <0.09%) (Freer *et al.* 2007) and all crops had K concentrations above the Maximum Tolerable Limit of 3 %DM (National Research Council 2005). The low Na and high K resulted in a grass tetany index that was above the risk level for cattle of 2.2 (Kempt and 't Hart 1957) and a K/(Na+Mg) index above the normal range suggested for sheep of 6 (Dove *et al.* 2016) in all wheat crops. High K combined with low Na significantly decreases the absorption of Mg from the rumen and can induce a Mg deficiency in sheep consuming adequate Mg (Suttle and Field 1969; Greene *et al.* 1983). Consideration of all results indicates wheat forages present a higher risk of induced Ca or Mg deficiency than barley or oats, however, this requires confirmation. All wheat crops were grown in NSW, and the barley and oat crops were grown in WA. Therefore, further research is required to distinguish the risks associated with crop type compared to location.

The patterns of response to supplements were consistent across five of the six experimental sites. Farm 6 was a clear outlier. Plant analysis indicates that ewes on this property were at a similar risk to those on other sites where wheat was sown, yet Ca concentrations in plasma were well above the deficient range in all groups and there were no responses to supplements. There is no obvious explanation for differences at this site.

The results do not provide strong evidence to support the second hypothesis that a mineral supplement designed to reduce DCAD, while providing Ca, Mg and Na, will be more effective than a supplement that has no influence on DCAD. Supplementation resulted in significant responses in urine pH, Ca and Mg in plasma and urine, and Ca fractional excretion on some farms. However no consistent differences between the two supplements were observed. The relevance to sheep of high DCAD in the crop forage and in supplement formulation requires clarification. High DCAD is usually associated with a decrease in bone Ca accretion and high urine pH. Others have reported a positive relationship between urine pH and DCAD (Takagi and Block 1991b; Grant *et al.* 1992). Provision of anionic salts to pregnant dairy goats and sheep reduced urine pH and elevated urine Ca, although these changes were not associated with greater bone resorption (Liesegang 2008). In these cited studies, DCAD treatments range from <-4 meq/100 g DM to >40 meq/100 g DM. Stacey and Wilson (1970) suggest aciduria could have a direct effect on increasing urinary Ca excretion by preventing renal reabsorption of Ca. By increasing urinary Ca excretion, low DCAD diets trigger the homeostatic mechanism of bone mobilisation and increased intestinal absorption of Ca to maintain plasma Ca (Block 1984; Scott *et al.* 1993; Wilkens *et al.* 2016). Wilkens *et al.* (2012) showed that bone Ca mobilisation is an important mechanism for maintaining Ca homeostasis in sheep. The lack of a clear difference in response to the different supplements may therefore be because sheep are less responsive to high DCAD diets. Alternatively, it may be that the New supplement with a lower DCAD did not make a large enough difference in the overall DCAD on ingested feed and minerals. Using farm 5 as an example, ewes were 4-5 weeks from parturition with an expected metabolizable energy requirement of 9.6 MJ ME/day (Ministry of Agriculture Fisheries and Food 1975). On the assumption that crops have an energy content of approximately 12 MJ ME/kg DM (Masters and Thompson 2016), feed intake would have been approximately 0.8 kg DM/day for each ewe. The DCAD of forage alone was estimated at 70.5 meq/100 g DM. Consumption of 0.8 kg forage along with 30 g/day of the New supplement would reduce the DCAD of total feed and supplement combined to 53 meq/100 g DM. Consumption of 0.8 kg forage with 30 g/day of the Standard supplement would reduce the DCAD of total feed and supplement combined to 67 meq/100 g DM. Therefore, the New supplement reduced the overall DCAD by 14 meq/100 g DM more than the Standard supplement, but the overall DCAD of 53 meq/100 g DM was still above experimental DCAD concentrations used to increase bone Ca resorption and lower urine pH under controlled experimental conditions.

In summary, these results indicate that either of the two supplements used can be effective in increasing Ca status in pregnant ewes. The results support the inclusion of Ca, Mg and Na in the supplement, although some caution is required as Ca supplements in pregnancy have also been reported to increase the risk of hypocalcaemia. Sykes (2007) asserts that hypocalcaemia is almost always due to the failure of the endocrine system to respond to an increased demand for Ca by increasing resorption from bone and gastrointestinal absorption rather than from a dietary deficiency of Ca. They argue that increasing plasma Ca through Ca supplements may be counterproductive as it works against the bodies homeostatic mechanisms. Conversely, Larsen *et al.* (1986) and Elias and Shainkin-Kestenbaum (1990) suggested that a diet deficient in Ca during the last month of pregnancy may facilitate hypocalcaemia in ewes and that Ca supplements such as limestone may be beneficial. Others also report that the risk of hypocalcaemia is not necessarily increased by feeding high Ca levels earlier in pregnancy (Sansom *et al.* 1982). Some of the uncertainty in response to supplements may be related to supplement type. The use of low DCAD supplements in the current experiment aimed to provide a Ca formulation that would improve, not compromise, the homeostatic response to increased Ca demand. Feeding excess anions (negative DCAD) shifts the systemic acid-base balance towards a mild metabolic acidosis (Goff and Horst 1998; Schonewille *et al.* 1999). This mild acidosis has a stimulatory effect on Ca regulating hormones and on the responsiveness of the small intestine, kidney and bone with a resultant increase in blood Ca concentration. Low DCAD diet in the study of Las *et al.* (2007) elevated ionic Ca in plasma by 13%.

Given the uncertainty around changing the Ca status of pregnant ewes, supplement testing on a commercial scale is required to establish whether the increase in Ca status resulting from supplementation decreases the risk of hypocalcaemia. In addition, research is required to determine whether a change in Ca status associated with lowering DCAD is more effective than feeding a standard high Ca supplement in ewes under acute Ca demand stress.

5 Conclusions and Recommendations

5.1 Conclusions

- Measurement of mineral concentrations in young crops indicates that a high proportion of the 17 commercial properties surveyed in experiment 1 had forage Ca (70%), Na (70%), Mg (18%) below published requirements and K (70%) above the published maximum tolerable level. All crops on all farms has a DCAD consistent with a decrease in the ewe's ability to mobilise Ca from bone. Analysis of samples collected from the ewes at the end of the grazing period indicated ewes on 94% of farms had alkaline urine and on >76% of farms Ca concentrations in the urine or Ca fractional excretion were in the marginal range. None of the flock average Ca concentrations in plasma were in the deficient range but 59% of the flocks contained some individual ewes with plasma Ca in the deficient range. The combination of low and unbalanced supply of minerals in forage and low or marginal status of Ca in urine and some plasma samples indicates ewes have an increased risk of direct or induced Ca while grazing young crops in late pregnancy. The low Na and high K content of crops may also pose a direct risk to production;

  Conclusion addresses objective 1 – Measure the changes in mineral status of ewes grazing winter crops on commercial farms during the last third of pregnancy)

- There was no strong evidence that ewes grazing crops were at a high risk of hypomagnesaemia. Forage analysis indicated 18% of crops contained less that published requirements and small proportion of flock-average concentrations of Mg (6%) and Na (18%) in plasma were in the deficient range. The marginal Mg in forage along with low Na and high K and high grass tetany index was expected to induce a low Mg status, so, while clinical Mg deficiency may be unlikely, the marginal status of Mg may predispose ewes to hypocalcaemia. When mineral supplement is fed to ewes grazing crops these should contain Mg;

- Risks of mineral imbalance appeared to be highest for wheat, then oats with lowest risk in grazing barley. However, due to the commercial constraints of this research, not all crops were grown at all locations and further research is required to confirm this conclusion;

- Low Ca in forage was associated with high urine pH and low Mg and Na and high K in forage were associated with low fractional excretion of Mg. The supply of Ca, Mg, Na and K in the forage therefore all contribute to the risk of hypocalcaemia and hypomagnesaemia. The strong negative relationship between Colwell K and forage Ca, Mg and Na indicates that Colwell K may also influence the susceptibility to mineral imbalance. Mineral status of forage and risk of metabolic disease are reflected in urine Ca, Mg and pH and fractional excretion of Ca and Mg;

  Conclusion addresses objective 2 – Determine if direct measures on mineral status in ewes can be used to predict the risk of metabolic disease).

- Ca status is improved by providing supplements containing Ca, Mg and Na. This was demonstrated by increases in Ca concentration in urine, plasma and Ca fractional excretion in supplemented ewes on five of the six experimental farms in experiment 2;

- The two mineral supplements used were both effective in improving Ca status. One of these was a standard industry supplement containing causmag, limestone, and salt (40:40:20), the other contained the same cations but as magnesium chloride, gypsum and salt (12.5:32.5:55);

- The supplements had no significant effect on condition score or ewe mortality within experiment 2. A response in condition score was not expected and group sizes were not large enough to detect differences in ewe deaths due to hypocalcaemia or hypomagnesaemia (typically <2%). This was an expected result as the experiment was designed to detect differences in Ca status as an indicator of risk of hypocalcaemia;

  Conclusion addresses objective 3 – Determined the production responses of ewe grazing winter crops to mineral supplements in late pregnancy).

- There are a number of management strategies that are now appropriate for road testing through national delivery networks apparent from this project and the review of the literature that accompanied it:

- In dry or drought periods where breeding ewes are supplemented with grain, Ca supplements should also be provided. High grain diets with a lack of Ca may result in Ca depletion and increased susceptibility to hypocalcaemia when grazing crops later;
- Pregnant ewes grazing young crops will usually have an unbalanced mineral intake and be at risk of low Ca status. The implications of this will vary depending on time on the crop and previous Ca history. Provision of a supplement containing Ca may improve status and reduce risk of deficiency
- There is an interaction between Ca, Mg, Na and K such that low Ca, Mg and Na combined with high K increase the risk of acute Ca deficiency. Therefore mineral supplements should include Na and Mg as well as Ca.
- Wheat crops grown in NSW (particularly those grown on high K soils) are at higher risk of mineral imbalance and Ca deficiency and are a high priority for management of mineral status.

Conclusion addresses objective 4 – Refined management strategies that must be followed to reduce the risk of ewe mortality and have these in a format to be road-tested by the national delivery networks such as Making More from Sheep and Bred Well Fed Well.

5.2 Recommendations

- The research to date has confirmed low Ca, Mg, Na and high K from grazed crops and low Ca status in ewes grazing crops. This would indicate a need for Ca supplements. However, there is still some uncertainty around the effectiveness of Ca supplements in pregnant ewes under an acute Ca stress. Sykes (2007) asserts that hypocalcaemia is almost always due to the failure of the endocrine system to respond to an increased demand for Ca rather than to low Ca status as indicated by plasma and urine Ca. In fact there are some suggestions that Ca supplements accentuate the endocrine failure, particularly in dairy cattle. This uncertainty remains to be resolved for pregnant ewes and requires large commercial scale experiments using high risk wheat crops where sheep numbers will be sufficient to significantly detect changes in potential and actual ewe and lamb mortality. For ethical reasons these experiments would require high labour input to detect and treat hypocalcaemic ewes through daily observations prior to death.

- From the research over two years, grazing wheat crops appeared to be highest risk. However, due to the commercial constraints of this research, not all crops were grown at all locations and the separate risks associated with either crop type or location could not be defined. It is recommended that different crops grown at the same sites be collected and analysed to allow definition of these risks. This could be carried out at low cost by collecting samples from crop variety trials.

- The two supplements tested in this project were both effective in improving Ca status of ewes under normal grazing conditions. It was expected that the low DCAD supplement would have been more effective through facilitation of Ca mobilisation from bone. It is recommended that these supplements also be tested under conditions of Ca stress and endocrinological challenge; a time when the low DCAD supplement would be expected to increase Ca supply and to improve Ca mobilisation from bone.

- Ca homeostasis in closely related to vitamin D status and supply. Vitamin D facilitates Ca mobilisation, absorption and retention. Crops are often grazed when available sunlight is low (vitamin D production is initiated by sunlight on the skin) and therefor vitamin D status is low. Future experiments should include vitamin D treatment as part of a factorial treatment design with mineral supplements.

6 Key messages

- Young vegetative crops are an excellent source of metabolisable energy (approximately 12 MJ/kg DM) and protein (crude protein 15- 37 %) for pregnant ewes.

- The forage is available at a time when nutrient requirements of reproducing ewes is high and ewes can maintain condition score while grazing forages with FOO of <200kg DM/ha. This is a much lower FOO than is required when grazing conventional pastures.

- Forage provides an unbalanced supply of minerals to grazing ewes with low Na, marginal Ca and Mg and high K. This imbalance results in low Ca status and marginal Mg status and an increased risk of metabolic disorders such as hypocalcaemia, hypomagnesaemia and pregnancy toxaemia.

- Mineral supplements will increase Ca and to a lesser extent Mg status. It remains to be confirmed that such supplements will overcome the endocrinological disturbance that causes hypocalcaemia under conditions of acute Ca stress. Supplements require testing on a commercial scale.

- Any mineral supplement used should contain Ca, Mg and Na

- Wheat crops grown in NSW are high risk forage. It is likely but not yet confirmed that wheat crops elsewhere will also be high risk. Sheep grazing oats and barley are also consuming an unbalanced mineral supply and should not be considered risk free.

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8 Appendix

8.1 Presentations

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Refshauge, G. Grazing cereals with demanding sheep - growth, health and welfare. March 17, 2016. Lachlan Valley Lamb Group, Research Update, Cowra, NSW, Australia.

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