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Managing metabolic disorders in pregnant ewes to improve ewe and lamb survival

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EXECUTIVE SUMMARY

Suboptimal levels of lamb survival are the largest contributor to reproductive wastage in Australian sheep flocks (Kleemann & Walker 2005), with an average 20% of lambs born dying mainly within 3 days of birth (Hinch & Brien 2014). This results in substantial loss of production, producer and industry income, and is increasingly being perceived as poor animal welfare. Improving lamb survival is therefore a priority for the industry.

Dystocia and the starvation-mismothering-exposure complex are generally the major causes of perinatal lamb mortality, and in combination account for approximately 80% of deaths. Management to reduce mortality therefore needs to address these causes to be effective. Clinical deficiencies of calcium (Ca) and magnesium (Mg) contribute to both ewe and lamb mortality, but their incidence is typically low. There is an increased risk of these disorders on lush, grass-based pastures, due to lower levels of calcium and magnesium in grasses than legumes. The role of sub-clinical deficiencies in lamb survival is less clear, but potential mechanisms include a reduction in muscle contractions and cervical dilation increasing the duration of parturition (leading to dystocia), appetite suppression, and through poorer temperature regulation and neuronal injury in newborns.

The aim of this project was to determine the extent of any reduction in calcium or magnesium during late pregnancy in ewes grazing common pastures; whether this was associated with increased lamb mortality; and whether mineral supplementation could be used to improve the calcium and magnesium status of reproducing ewes, reducing the incidence and consequences of sub-clinical hypocalcaemia and hypomagnesaemia, in particular, lamb mortality.

A review of the literature found evidence indicative of potential mechanisms whereby subclinical calcium and magnesium deficiencies might reduce lamb survival. Monitoring of 16 lambing flocks across NSW, SA, VIC and WA in 2016 demonstrated that a third of flocks had more than 20% of ewes with below adequate calcium or magnesium concentrations a week before lambing, when grazing typical pastures. An intensive pen study with twin-bearing ewes showed that although the calcium and mineral status of ewes did not significantly alter the duration of parturition (although there was a statistical trend, $P < 0.10$), supplementation with minerals did improve energy regulation in the ewe, potentially enabling the ewe to maintain health in less optimal conditions. Furthermore, mineral supplementation improved the immune response in both ewes and lambs, proving a mechanism through which lamb survival might be altered. Lamb weight at 4 weeks of age was also improved by supplementation. The pen study did not contain sufficient ewes to measure lamb survival, so the impact of supplementation was tested in grazing ewes on commercial properties.

In 2017, a replicated study used 5 flocks (across NSW, SA, and WA) with control and supplemented groups. The supplement offered provided Ca, Mg and sodium (Na) in a form designed to reduce the dietary cation-anion difference (DCAD), and was similar in composition to that offered in a recent experiment on grazing cereals,

which was readily consumed by sheep and improved their Ca and Mg status (Masters et al. *in press*). The mineral supplement was only consumed in amounts that could be effective by two of these flocks, but did not improve lamb survival in any flock. The study was repeated in 2018 on another flock, comparing both standard (1:1:1 lime, causmag and salt) and the low DCAD supplements, to test whether the form of the supplement was an issue. In this study, both supplements were readily consumed near the target level of 20 g/ewe/day, but had minimal effects on plasma and urine calcium and magnesium levels, and did not improve lamb survival. This may have been due to variable intake of the supplement, type of pasture or level of grain feeding, since metabolic levels were manipulated in the previous pen study where sheep were individually fed known amounts of Ca and Mg.

It is concluded that many late pregnant ewes in Australian grazing flocks are sub-clinically deficient in calcium and magnesium. While the supplementation studies undertaken on commercial flocks grazing common pastures did not show any improvement in lamb survival, supplementation was shown to improve the metabolic status of ewes and the immunity of their lambs under a controlled feeding situation, which can be expected to reduce the risk of clinical metabolic disorders and other contributors to lamb mortality. The low cost of supplementation warrants their use as a possible preventative measure.

1. INTRODUCTION/HYPOTHESIS

In Australia, an average 20 to 30% of lambs born die within 3 days of birth (Hinch & Brien 2014), although the level varies considerably between farms, regions and years (Oldham *et al.* 2011; Curtis 2014; Hinch & Brien 2014; Paganoni *et al.* 2014). Perinatal lamb mortality is the largest source of reproductive wastage in sheep (Kleemann & Walker 2005), causing significant financial loss, with an increasing risk of being perceived as poor animal welfare. Identifying management which could improve lamb survival is therefore a priority for the sheep industry.

Dystocia is one of the key causes of lamb mortality in the perinatal period (Hinch & Brien 2014), so is a key area where there may be potential for management intervention. Calcium deficiency may be implicated in higher rates of dystocia (Silva & Noakes 1984) and therefore reduced lamb survival. There is some evidence, unsurprisingly, that ewes recovering from clinical hypocalcaemia will have lower lamb survival (Nosdøl & Waage 1981). Supplementation with Mg has reduced piglet mortality (Trawńska *et al.* 2013; Zang *et al.* 2014). However, it is unknown whether sub-clinical deficiencies of either Ca or Mg could have an impact on lamb survival.

That acute hypocalcaemia and hypomagnesaemia exist in Australian sheep flocks is well documented (Herd 1966b; Caple *et al.* 1988b), however the occurrence is usually in a small percentage of the flock. Clinical disease usually occurs when sheep are grazing winter or spring pastures or young vegetative dual-purpose cereal crops. These forages may be marginal in Ca and Mg but with a high dietary cation-anion difference (DCAD) and high concentrations of potassium (K) and nitrogen (N) (Grant *et al.* 1988; Masters & Thompson 2016). The low sodium (Na) in grazed vegetative wheat further predisposes grazing sheep to Mg deficiency (Dove 2007; Dove & McMullen 2009; Dove *et al.* 2012; Dove & Kelman 2015; Dove *et al.* 2016; Masters & Thompson 2016). Therefore, in flocks that show clinical signs, it is likely that a high proportion of the sheep in that flock have a low Ca and Mg status. The potential for sub-clinical deficiencies on the wider range of common pastures that lambing ewes typically graze is, however, unknown.

The aim of this project was to evaluate the potential for sub-clinical calcium and magnesium deficiencies in ewes to influence the rate of lamb survival. The studies aimed to identify whether sub-clinical deficiencies occurred in ewes grazing common pastures, whether this was associated with reduced lamb survival, and whether mineral supplementation could therefore improve lamb survival.

2. LITERATURE REVIEW

Below is a draft review which will be submitted for publication in a scientific journal, most likely *Animal Production Science*. The review was written by Marie Bhanugopan, Janelle Hocking Edwards, Michael Friend, Serina Hancock, Kate Loudon, David Masters, Shawn McGrath, Peter McGilchrist, David Miller and Gordon Refshauge.

Causes of lamb mortality in grazing sheep

Dystocia and the starvation-mismothering-exposure complex are generally the two major causes of lamb mortality (Kenyon *et al.* 2003; Geenty *et al.* 2014). A recent analysis of 26,630 birth records and 3,198 necropsy records (birth to 5 days of age) from the Sheep CRC Information Nucleus Flock, indicated that 48% of the lambs that died were either dead from causes associated with difficult and prolonged birth (still birth, dystocia and birth injury), approximately 25% died of starvation and mismothering, approximately 10% were dead *in utero*, and less than 1% died from infection (Refshauge *et al.* 2016). The results of autopsies of 4417 lambs in WA indicated that 46% were caused by starvation, 18% by dystocia, 9% by congenital defects and 9% by infection. Management to improve lamb survival therefore needs to target the causes of either dystocia, and/or the starvation-mismothering-exposure complex if large improvements are to be achieved. For mineral imbalances to be implicated in the death of the majority of lambs, then it would need to occur through dystocia leading to difficult or prolonged birth processes, reduced lamb viability (independent of dystocia issues) or via complications with milk let down, colostrum quality and milk yield.

Birthweight is the single largest determinant of lamb survival in the first few days of life and is influenced by birth type and lamb gender (Kelly 1992; Schreurs *et al.* 2010) as well as maternal nutrition during pregnancy (Oldham *et al.* 2011; Paganoni *et al.* 2014). Irrespective of the source of variation in birthweight, survival is lower in very small lambs, where death is due to the starvation-mismothering-exposure complex, or very big lambs which are more likely to die due to dystocia (Hinch & Brien 2014). Feeding ewes to maximise the number of lambs born within the desired weight range will reduce lamb mortality (Mukasa-Mugerwa *et al.* 1994; Oldham *et al.* 2011), but the majority of lambs which die are within the optimal birthweight range, so factors in addition to birthweight are important.

Nutritional supplements may improve lamb survival independent of birthweight (Hinch *et al.* 1996) and deficiencies of many specific nutrients such as iodine (I), zinc (Zn), selenium (Se), vitamin E (vit E) and copper (Cu)

(Masters & Fels 1980; Judson & McFarlane 1998; Liu *et al.* 2014) compromise the outcome of pregnancy directly, or disrupt the ability of the ewe to deal with environmental or physical stress. For example, I deficiency will reduce thermogenesis and increase susceptibility to cold stress (Caple *et al.* 1985) and Se deficiency will depress immune function (Hogan *et al.* 1993). Along with this, Ca and Mg deficiency will increase ewe mortality during pregnancy and deficiencies in a range of nutrients will cause abnormal fetal development (Robinson *et al.* 2002).

Causes and consequences of prolonged and difficult birth

Dystocia is defined as a difficult birth (Arthur 1975) or birth process due to a long, unassisted parturition or a prolonged or severe delivery requiring assistance (Zaborski *et al.* 2009), the causes for which are maternal or fetal in origin. Left unassisted, dystocia directly compromises dam and neonatal survival (Arnold & Morgan 1975; Smith 1977), while for survivors lasting implications include reduced progeny vigour (Dwyer 2003; Dwyer & Bünger 2012; Fonsêca *et al.* 2014), impaired maternal instinct, delays in lamb development, reduced dam fertility and more stillbirths in subsequent pregnancies (Fogarty *et al.* 1992; Waage & Wangensteen 2013; Dwyer 2014).

The fetal origin of dystocia is limited to fetal oversize, malpresentation and congenital deformities, while the maternal origins of dystocia are due to issues affecting the expulsive forces (Arthur 1975) or pelvic constriction, including the mismatch between the fetal size and pelvic shape (George 1976; McSporran & Fielden 1979; Cloete *et al.* 1998).

The expulsive forces affecting uterine inertia can be impaired due to a ruptured uterus, uterine torsion, defects of the myometrium, excessively large fetus or a large number of fetuses, hormonal imbalance, premature birth or environmental disturbance, and a possible role for Ca and Mg deficiencies has also been suggested (Arthur 1975; Lane *et al.* 2015).

Dystocia may occur following insufficient dilation, such as when observed with uterine torsion. Impaired dilation affects the cervix, vagina and vulva, but none of these appear to be affected directly by mineral nutrition (Arthur 1975). Incomplete dilation of the cervix varies from year to year, with the possibility of an environmental cause impacting on the endocrine system (Arthur 1975). Hindson (1961) reported an increased incidence of incomplete dilation in seasons when grass was abundant, compared to a lower incidence in years of very dry seasons and lower twinning rates. In this example, unfortunately, pasture abundance is confounded with litter size, such that either grass dominant forages (i.e. high K, low Na, low Ca, low Mg) or the higher mineral nutrient demand of twin and triplet pregnancies (CSIRO 2007), or both are involved with incomplete dilation.

The probability of dystocia has been shown to increase when maternal heat loss was greater in the fortnight prior to parturition, reducing lamb viability (Everett-Hincks & Dodds 2008). Greater heat loss in the three days prior to parturition increased losses to dystocia and starvation/exposure and these effects were more important

than the weather that occurred during lambing. These authors concluded that heat loss was affecting maternal energy balance. Cold weather may have another effect, which is to increase K and decrease Mg uptake by the root system of the plant (Elliott 2009). An increased dietary intake of K may lower the absorption of Ca (Bhanugopan *et al.* 2015), which may also partly affect parturition duration.

Prolonged parturition increases the risk of hypoxia (a deficiency of oxygen), hypoxaemia (low blood flow), hypercapnia (elevated carbon dioxide in the blood) and metabolic acidosis leading to death due to hypoxic ischemic encephalopathy (brain injury caused by oxygen deprivation) (Dutra & Banchero 2011). Asphyxia at birth is a key factor affecting lamb mortality and many of the factors typically associated with lamb mortality affect the duration of birth including birth weight, placental size, litter size, sex, lamb body dimensions and dam breed (Dutra & Banchero 2011; McHugh *et al.* 2016). The metabolic consequences of depleted oxygen include a rapid depletion of high-energy phosphate reserves, including adenosine triphosphate; accumulation of lactic acid and the inability to maintain cellular functions. Disrupted cellular functions include transcellular ion pumping, resulting in intracellular accumulation of Na, Ca and water (Perlman 2006).

Mishra & Delivoria-Papadopoulos (1999) suggest the susceptibility of the fetal brain to hypoxia increases as the brain develops closer to term. The factors affecting the susceptibility of the neonatal brain to hypoxia may include the lipid composition of the brain cell membrane; the rate of lipid peroxidation; the presence of antioxidant defences and; the development and modulation of the excitatory neurotransmitter receptors, such as the N-methyl-D-aspartate (NMDA) receptor, the intracellular Ca^{++} and intranuclear Ca^{++} influx mechanisms (Mishra & Delivoria-Papadopoulos 1999). Both lipid peroxidation and hypoxia can affect phosphorylation mechanisms and the activity of sodium-potassium adenosine triphosphatase, which supports cellular ionic gradients. Between 6 and 48 hours after the primary hypoxic event, a secondary cerebral energy failure appears to occur (Perlman 2006) and this will be associated with a rapid depletion of ATP. Ischemic neurons first appear 24 hours post-partum and increase in number between 48 hours and 5 days (Dutra *et al.* 2007). A potential role for Mg in neuroprotection is described later in this review.

Calcium deficiency in grazing sheep and potential role in lamb mortality

Hypocalcaemia, frequency, timing and physiological state

The normal reference range for plasma Ca is 8.5 to 10 g/dl, and a sudden decrease in the concentration may result in hypocalcaemia. In clinically hypocalcaemic recumbent sheep, serum Ca concentrations may be below 1.0 mmol/L. Without appropriate therapy, the condition develops to coma, and death follows 24 to 48 hours after onset of recumbency (Treacher & Caja 2002; Scott 2013).

Hypocalcaemia may lead to the development of secondary diseases such as an increased incidence of dystocia, retained placenta, displaced abomasum, and uterine prolapse in dairy cows (Curtis *et al.* 1983b; Grohn *et al.* 1990). In ewes with experimentally induced hypocalcaemia, lower uterine activity during parturition was linked to an increase in dystocia (Silva & Noakes 1984). Nosdøl & Waage (1981) reported that ewes that recovered from hypocalcaemia had increased lamb loss due to premature birth. Caple *et al.* (1988b), reported that sub-clinical hypocalcaemia in ewes resulted in lower lamb survival rates. Thus, clinical and sub-clinical hypocalcaemia could be predisposing factors to dystocia and uterine inertia increasing the incidence of lamb mortality.

Hypocalcaemia is widespread in Australia. In the GHD Pty Ltd (2015) report, the percentage of flocks affected was assumed to vary from 0.2% to 0.4% depending on rainfall zone and sheep class. This estimate would appear very conservative when compared with historical estimates of 100,000 – 300,000 pregnant ewes dying from hypocalcaemia each year in Victoria alone (Caple *et al.* 1988b).

Hypocalcaemia occurs due to transfer of Ca to the growing fetus and milk resulting in a negative maternal Ca balance during late pregnancy and early lactation (Braithwaite *et al.* 1969; Braithwaite *et al.* 1970; Sansom *et al.* 1982). Susceptibility increases from about 6 weeks prior to lambing to about 3 to 4 weeks after lambing. Most cases occur in the last few weeks of gestation when the fetal skeletons are mineralising (Nosdøl & Waage 1981; Bozos *et al.* 2011; Baird & Pugh 2012a; Scott 2013). The Ca requirement of twin-bearing ewes is higher than single-bearing ewes, making them more susceptible to hypocalcaemia (Treacher & Caja 2002; Suttle 2010). Older ewes are also more susceptible due to depletion of Ca reserves in previous pregnancies (Sargison 2009).

Metabolic mechanisms for calcium

In adult animals, 99% of total body Ca is found in the bone, and the remaining 1% is within intracellular and extracellular fluid (Suttle 2010). Ca plays an important role in blood clotting, skeletal and smooth muscle contraction, in the release of neurotransmitter in neuromuscular junction and acts as second messenger and as cofactors in number of metabolic functions within the body (Goff 1989; Cunningham & Klein 2013). Low Ca concentrations allow Na to enter nerve cells, which increases nerve irritability resulting in spontaneous

contractions and fasciculations of skeletal and smooth muscle (Coffman 1980; Gordon 1989; Breazile 1994). In muscles, lack of Ca prevents initiation of muscle contractions that are initiated by Ca release from the sarcoplasmic reticulum and mechanically effectuated through Ca binding to troponin C, which enables formation of actin-myosin complexes (Iggo 1983; Gordon 1989; Breazile 1994). Troponin C has 4 receptor sites, 2 of which have high affinity for Ca and 2 for which Ca and Mg compete (Gordon 1989). The role of the specific Ca binding site is essential in the process of muscle contraction (Ward *et al.* 2000), whereas Mg plays a modifying role at this level (Gordon 1989). Hypomagnesemia potentiates the effects of hypocalcaemia (Smith & Wagner 1985) because it increases the release of acetylcholine at neuromuscular junctions (Gordon 1989). Expressions of over-excitability of the peripheral nerves or CNS are also caused by disturbances in Ca, Mg, or K concentrations (Kimura 1989; Jableck 1993).

Sub-clinical hypocalcaemia

The consensus is that an animal with a blood Ca concentration between 8.0 mg/dl (2.0 mmol/l) and 5.6 mg/dl (1.4 mmol/l) and not showing clinical signs, can be considered as having sub-clinical hypocalcaemia. Although cows with sub-clinical hypocalcaemia exhibit no observable clinical signs, they do manifest less specific symptoms, such as loss of appetite and muscle weakness, and are predisposed to perinatal diseases, including ketosis, dystocia, placental retention, displacement of the abomasum, uterine prolapse, endometritis, and mastitis (Curtis *et al.* 1983a; Risco *et al.* 1984; Reinhardt *et al.* 2011; Martinez *et al.* 2012). Both hypocalcaemia and sub-clinical hypocalcaemia suppress rumen and abomasal motility (Huber *et al.* 1981; Daniel 1983) and appetite (Field *et al.* 1975; Beede 1992; Underwood & Suttle 1999), thereby compromising dry matter intake and predisposing animals to pregnancy toxaemia. Hypocalcaemia also predisposes animals to postpartum diseases such as mastitis and metritis which suppress appetite (Martinez *et al.* 2012), also predisposing animals to pregnancy toxaemia (Martinez *et al.* 2014).

In sheep, low blood Ca has been associated with prolapse in pre-parturient ewes not showing signs of hypocalcaemia. Dietary Ca and/or factors affecting its dietary absorption or bone resorption were contributing factors (Stubblings 1971; Sobiraj *et al.* 1986). In Saudi Arabia and Iraq, incomplete cervical dilation is an important cause of dystocia and affected ewes do respond to parenteral sources of Ca or prostaglandin PGF₂ α (Majeed and Taha 1989; Ali 2011). The literature on hypocalcaemia, however, focusses on clinical deficiencies and ewe health and it remains unclear whether sub-clinical hypocalcaemia is a cause or contributing factor in dystocia.

Dietary factors that influence hypocalcaemia

A ewe with a single fetus or lamb grazing a temperate pasture with dry matter digestibility of 74% and maintaining a maternal weight of 50 kg requires a dietary Ca concentration of 0.40% DM in late pregnancy, and 0.38% DM during lactation (CSIRO 2007).

Some difference is apparent in the literature in regard to the role of a diet deficient in Ca in the occurrence of hypocalcaemia. Larsen *et al.* (1986) and Elias & Shainkin-Kestenbaum (1990) suggested that a diet deficient in Ca during the last month of pregnancy may facilitate hypocalcaemia in ewes as a constant dietary intake is required to offset the loss of Ca to the fetus. However, Sykes (2007) asserts that hypocalcaemia is almost always due to the failure of the endocrinology system to respond to increased demand by increasing resorption from bone and gastrointestinal absorption rather than from a dietary deficiency of Ca.

It is therefore not surprising that divergent views exist on the supplementation of ewes during late-pregnancy. Larsen *et al.* (1986) suggests that Ca supplements such as limestone may be beneficial, however Sykes (2007) argues that such an approach may be counterproductive as it works against the bodies homeostatic mechanisms. In dairy cows, an approach of feeding low Ca diets during late pregnancy is commonly used to stimulate homeostatic mechanisms well before calving. However, under different circumstances, both high and low dietary Ca have been shown to prevent milk fever (McNeill *et al.* 2002). Goff & Horst (1997a) reported that dietary Ca intake did not have a significant effect on the incidence of milk fever in dairy cows, and these researchers suggested the benefits of a low dietary Ca preventing hypocalcaemia observed in some studies may relate to a concurrent reduction in dietary K rather than low dietary Ca *per se*. If this relationship is similar in sheep, the high K concentration of wheat forage (Dove & McMullen 2009) and other winter and spring forages (Jacobs & Rigby 1999) may increase the risk of hypocalcaemia in reproducing ewes grazing these forages.

An approach recommended by Robinson *et al.* (2002) is provision of a low Ca diet to ewes prior to the large increase in demand during late pregnancy to stimulate the homeostatic mechanism involving PTH. Dietary Ca is then increased as lambing approaches. However, the risk of hypocalcaemia is not necessarily increased by feeding high Ca levels earlier in pregnancy (Sansom *et al.* 1982), and the timing of changes to dietary supplements may not be practical in the extensive sheep industry (Sykes 2007) where ewes typically lamb over a period of in excess of 4 weeks. Furthermore, Wilkens *et al.* (2012) reported that goats, but not sheep, are able to increase Ca absorption from the intestine in response to dietary restriction in Ca. Restricting dietary Ca for late-pregnant ewes under Australian conditions may therefore not be appropriate for preventing hypocalcaemia in sheep.

Excess phosphorus (P) in the diet reduces bone resorption and is a predisposing factor in hypocalcaemia. A dietary Ca:P ratio in the range of 1.4:1.0 to 1.0:1.0 is recommended (Treacher & Caja 2002).

Mg status of ewes may be important for Ca homeostasis. Ca absorption in the gastrointestinal tract is increased by increasing the Mg intake of sheep (Kozakai *et al.* 2002). Schneider *et al.* (1985) considered this result indicated that either synthesis of and/or end organ response to vitamin D is impaired in Mg deficient sheep. Goff (2006) concluded that hypomagnesaemia both reduces PTH secretion in response to hypocalcaemia and reduces tissue sensitivity to PTH. Mg intake and status of sheep is therefore important for Ca homeostasis, and it is unsurprising that hypocalcaemia often occurs in the later stages of hypomagnesaemia. Herd (1965) reported that hypocalcaemia did not occur when serum Mg levels were normal, although a combined hypocalcaemia/ hypomagnesaemia in the lactating ewe is not always sufficient condition for clinical grass tetany (Herd 1966b). It is not clear why, in some cases of high ewe mortality observed in northern NSW associated with ewes grazing wheat crops and other lush pastures, a concurrent hypermagnesaemia was observed with hypocalcaemia in affected ewes; although the same report noted that some sick ewes responded to intravenous infusion of magnesium sulphate (Blumer *et al.* 1939), implying a Mg deficiency. A possible explanation is that if Mg intake is high enough to cause an increase in blood pH, this could negatively impact Ca homeostasis (Suttle 2010).

Although there are some studies indicating ruminants consuming a high potassium diet increase or show no change in absorption of Ca (Newton *et al.* 1972; Greene *et al.* 1983a; Grings & Male 1987; Fredeen 1990), the weight of evidence suggests that high K intake is a risk factor in the development of hypocalcaemia. High K will increase the cation-anion difference and may also reduce Mg absorption. Studies have shown that K competitively inhibits Ca accumulation by intestinal slices *in vitro* (Schachter *et al.* 1960). Greene *et al.* (1983a) and Wylie *et al.* (1985) observed that Ca absorption tended to be reduced when high levels of K were fed. Dietary K levels of greater than 30g/kg DM resulted in decrease in Ca absorption and retention in Welsh mountain sheep (Phillips *et al.* 2006). Similar results were also found by (Bhanugopan *et al.* 2015), where an increase in K in the diet led to a decrease in Ca absorption index from the intestine.

Reducing the dietary cation-anion difference (DCAD, calculated as $[(Na+K)-(Cl+S)]$, in mEq) reduces blood pH, promoting Ca absorption from the small intestine and resorption from bone (McNeill *et al.* 2002). This has been exploited in dairy cows to prevent hypocalcaemia by feeding anionic supplements to dry cows during the last 3 weeks before calving to reduce the DCAD of the diet (McNeill *et al.* 2002; Goff 2006). In pasture-based dairy systems, the results of using anionic salts to prevent hypocalcaemia have been less convincing, perhaps due to the high DCAD of pastures, and particularly the high K content (McNeill *et al.* 2002; CSIRO 2007).

A change to lush pasture with a low dry matter content may reduce Ca absorption; an average dry matter concentration of 12.8% DM (range 8.1 - 19.9% DM) was reported in mixed pastures associated with the occurrence of hypocalcaemia in late-pregnant ewes, perhaps due to reduced Ca absorption associated with increased rate of flow of digesta (Larsen *et al.* 1986). Ewes grazing such pastures have difficulties to maintaining adequate concentrations of Ca in plasma (Grant *et al.* 1988).

The vitamin D status of ewes has been implicated in the development and prevention of hypocalcaemia. Seasonal deficiency in vitamin D has been reported in ewes and lambs in south-eastern Australia along with improved lamb survival in lambs supplemented with vitamin D at marking (Caple *et al.* 1988b). However, a short period of reduced *in vivo* synthesis is unlikely to be of importance because body stores of vitamin D in adult animals can make good a dietary deficiency for several months (CSIRO 2007).

Magnesium deficiency in grazing sheep and potential role in lamb mortality

Hypomagnesaemia, frequency and physiological state

Clinical hypomagnesaemia is also known as grass tetany, grass staggers, lactation tetany, wheat pasture poisoning and winter tetany (Berger 1992). Before tetany occurs in sheep, ewes appear nervous or excited, with trembling, particularly in the facial muscles; onset is rapid and the disease can result in death if untreated (Treacher & Caja 2002).

Clinical hypomagnesaemia is less common in ewes than cows, perhaps because of greater Mg absorption and retention in sheep (Thompson & Reid 1981; CSIRO 2007). While the incidence is generally low in sheep, hypomagnesaemia can be a problem in individual flocks at pasture at the peak of lactation, in the first 4 to 8 weeks after lambing (Herd 1965; Treacher & Caja 2002). It is more common in older ewes rearing twin lambs, particularly if underfed (Treacher & Caja 2002). This increased susceptibility may relate to reduced bone turnover rates in older sheep (Sykes 2007) and higher loss of Mg associated with higher milk production in ewes with multiple lambs (Snowder & Glimp 1991). Producer-reported cases of grass tetany associated with grazing dual-purpose wheat in southern NSW ranged from 0.3-2.0% of ewes in flocks where hypomagnesaemia was reported, with 24% of flocks reporting some level of losses to grass tetany when grazing wheat (McGrath *et al.* 2013).

Metabolic mechanisms

The skeleton contains approximately 60 - 70% of the Mg in the body, muscle 25% and extracellular space 1% (CSIRO 2007). Mg is the most abundant intracellular divalent cation and is a cofactor for many enzymes involved

in oxidative phosphorylation and the metabolism of carbohydrates, lipids, proteins and nucleic acids (CSIRO 2007). Non-enzymatic functions include influencing folding of ribnucleotide chains, cell membrane integrity and influences on muscle contraction through exchanges with Ca (Suttle 2010). Mg is also associated with Ca homeostasis through involvement in the interaction between PTH with its receptors on bone and kidney cells (McDonald *et al.* 2011). The important role of extracellular Mg in moderation of nerve impulses and neuromuscular transmission has important implications for animal health; a reduction in blood Mg can reduce the concentration of Mg concentration in cerebrospinal fluid; if this level falls below 0.5 mmol/l hypomagnesaemic tetany may result (CSIRO 2007). Ruminants only have a small body reserve of Mg, most of which is bound in the skeleton and can only be released with bone resorption, and the animal is therefore reliant on dietary supply of Mg (Sykes 2007).

The Mg content of milk is low (4 mmol/L); however, loss of Mg represents a continuous drain on maternal reserves even when a deficiency occurs (Suttle 2010), and requirements of lactating ewes (0.12 % DM) are higher than for dry adult sheep (CSIRO 2007). Sheep cannot readily mobilise Mg stored within the body, so factors that reduce absorption of Mg to meet immediate demand, including reduced dietary supply of Mg, may cause hypomagnesaemia (Sykes 2007). Furthermore, even when Mg levels in forage appear adequate a deficiency could still occur (Swerczek 2008).

Secondary disorders

Hormonal control of carbohydrate metabolism

Mg is an essential cofactor for all metabolic reactions utilizing high energy phosphate bonds thus is critical for the rate-limiting enzymes related to carbohydrate metabolism and in the regulation of certain endocrine secretions. Mg in the body is not specifically regulated by any one hormonal factor however its balance and transport is influenced by many hormones including noradrenaline, adrenaline, insulin and glucagon.

Mg deficiency results in poor glycaemic control and insulin resistance by impairing both insulin secretion and its action on peripheral tissues (Suarez *et al.* 1995). Hypomagnesaemia in sheep fed diets low in Mg and high in K resulted in both the depression of insulin-induced glucose disposal and glucose-induced insulin secretion (Matsunobu *et al.* 1990).

Immunity transfer

Colostrum provides immunological protection to the neonatal lamb through the transfer of immunoglobulins (Nowak & Poindron 2006), and there is some evidence to suggest that lamb mortality is correlated with low colostrum immunoglobulin concentrations (Tabatabaei *et al.* 2013). Mg has been shown to have a key role in immune response (Laires & Monteiro 2008), however its importance to immunity transfer may be a mechanism by which it is important for neonatal lamb survival. Studies in pigs have shown that supplemental Mg fed to sows can increase the number of piglets born, born alive and weaned, and the weight gain of litters to weaning (Zang *et al.* 2014), and decrease mortality rates of newborn pigs (Trawńska *et al.* 2013), although other studies have reported a linear decrease in survival and litter weight at weaning as Mg supplementation levels increased (Hou *et al.* 2014). Immunoglobulin A (IgA) levels in sow colostrum and milk increased linearly with increasing Mg supplementation (Hou *et al.* 2014).

Sub-clinical magnesium deficiency

The consensus is that an animal with a blood Mg concentration below 2.3 mg/dl (0.9 mmol/l) and 1.7 mg/dl (0.7 mmol/l), and not showing clinical signs, can be considered as having sub-clinical hypomagnesemia.

Hypocalcaemia may facilitate pregnancy toxemia by exerting additive depressive effects upon the glucose homeostatic system (Schlumbohm & Harmeyer 2003). The link between Mg and insulin suggests that hypomagnesaemia may also have a role in pregnancy toxemia. By modulating insulin sensitivity and depressing insulin release, low Mg may further compound the glucose imbalance in ketosis (Hove 1978).

Hypomagnesaemia and lamb mortality

In addition to anoxia, non-lethal hypoxic damage caused during labour contributes to mortality indirectly. Neuroprotectants limit or slow the neuronal damage during hypoxia. Mg is a neuroprotectant as it acts to limit cell apoptosis through the reduced production of pro-inflammatory cytokines and free radicals after hypoxia-ischemia (Marret *et al.* 2007). Ca and Mg also play a role in temperature regulation of newborns. In rodents it has been found that low Ca and Mg reduce core body temp and UCP2 expression (a key protein for thermogenesis due to its role in brown adipose tissue metabolism) in the offspring (Gouvern *et al.* 1993; Zemel 2004).

Dietary interaction of magnesium with other mineral/nutrients

High potassium content of the diet is a risk factor for hypomagnesaemia as it reduces Mg absorption from the rumen and lowers Ca in serum (Greene *et al.* 1983c; Khorasani & Armstrong 1990; Schonewille *et al.* 1999a). Feedstuffs with K concentration as low as 2 to 3% DM may be tetany prone (Greene *et al.* 1983c). The depressive effect of K on Mg absorption may be due to an increase in rumen pH associated with higher K intake and the increase in salivary buffering that occurs with grazing (Khorasani & Armstrong 1990). K intakes to those

comparable to sheep grazing spring pastures have been shown to be sufficient to produce tetany at lower Mg intake (Suttle & Field 1969). The degree to which high ruminal K antagonises Mg absorption is dependent on the ruminal Mg concentration (Martens & Schweigel 2000). At low ruminal Mg levels, the depressive effect of ruminal K levels on Mg absorption is much more pronounced than when rumen Mg concentration is high (Ram *et al.* 1998; Martens & Schweigel 2000). Thus supplementing with high levels of Mg can overcome the negative effect of a high K diet on ruminal Mg absorption (Ram *et al.* 1998; Martens & Schweigel 2000).

Increased production of aldosterone due to sodium deficiency results in a decrease in salivary Na and an increase in salivary K as a result of increased production and secretion of aldosterone. This subsequently results in a change of the ratio of these minerals in ruminal fluid (Martens & Schweigel 2000). While it is accepted that the high ruminal K:Na ratio has a negative effect on Mg absorption, there remains uncertainty about the effect of variation in the relative concentration of K and Na in the diet of ruminants on Mg absorption (Sykes 2007).

Dietary protein is converted to ammonium in the rumen by ruminal microbes (Annison *et al.* 2002). Fresh forage diets can result in high ammonia concentrations in the rumen (Brookes & Nicol 2007), and ruminal ammonium levels to 30 - 70 mmol/l, equivalent to those from feeding young grasses, have been shown to reduce Mg absorption from the rumen, with the effect being additive and independent of K (Care *et al.* 1984). The effects of raising rumen ammonia concentrations on Mg absorption are inconsistent and transient (Suttle 2010), and may relate to high levels of K in pasture also containing high protein levels, rather than high protein concentration *per se* (Sykes 2007). High dietary protein, including non-protein nitrogenous compounds, has been implicated as the cause of an anionic imbalance, contributed to by both the dietary intake and the endogenous production of nitrate by bacteria in the gastrointestinal tract of ruminants (Swerczek 2008). This may produce excessive anionic ions which are subsequently neutralised by cations, causing the “washing out” effect of essential cations, including Mg, Ca and Na in urine, faeces and milk (Swerczek 2008), although no experimental evidence of this effect has been presented.

A high water content in forages can restrict dry matter intake of livestock (Clark & Woodward 2007). A low dry matter concentration in forage may be linked with metabolic diseases such as hypocalcaemia and hypomagnesaemia, by reducing the absorption of key minerals as a result of increased rate of flow of digesta (Larsen *et al.* 1986; Foster *et al.* 2007).

The dietary cation-anion difference of a diet affects the acid base balance of the body. Rations with a low or negative DCAD shift the acid-base to a metabolic acidosis (Stewart 1983). In an experiment with periparturiant

dairy cows, the concentration of plasma Mg increased as dietary DCAD decreased (Roche *et al.* 2003). The result suggests low DCAD inducing a metabolic acidosis is responsible for the increased absorption of Mg (Roche *et al.* 2003). No similar work has been conducted in sheep.

Indices have been developed to predict the risk of grass tetany caused by interactions between Mg, Ca and K. The tetany index or tetany ratio, originally developed in the Netherlands, provides an indication of whether cattle grazing a particular pasture are at greater risk of developing grass tetany. The index is calculated from the following equation, with all concentrations expressed as a % diet DM (Kempt & 't Hart 1957):

$$\frac{\% \text{ K} \times 256}{\% \text{ Ca} \times 499 + \% \text{ Mg} \times 823}$$

$$\% \text{ Ca} \times 499 + \% \text{ Mg} \times 823$$

The original report noted that incidence of tetany in cows increased rapidly when the index exceeded 2.2 (Kempt & 't Hart 1957), and this ratio has been used in a number of studies to rate the tetany risk associated with various forages (Mayland *et al.* 1976; Reeve & Sharkey 1980; Jefferson *et al.* 2001). The tetany index exceeded 3 at the time when tetany occurred in cows grazing a wheat and rye mixed pasture in a US report (Bohman *et al.* 1983), but also exceeded 2.2 at other times during the same experiment; despite this tetany was only observed close to the time of parturition (Bohman *et al.* 1983). Livestock may therefore need to be in a physiological state more susceptible to tetany for the tetany index to be relevant. The increase in the tetany index at the time when tetany occurred was due to increasing K concentration, with Mg and Ca in forage not changing appreciably (Bohman *et al.* 1983). In Australian studies with lambs, the tetany index in wheat forage during the grazing period exceeded 2.2 in all experiments (Dove & McMullen 2009; Dove *et al.* 2012), however, no cases of tetany were reported, although an increase in live weight gain in response to mineral supplementation was observed in the earlier study. As only young sheep were grazing wheat the risk of tetany was lower than for lactating ewes. Sheep exhibit higher absorption and retention of Mg than cattle (Thompson & Reid 1981), so the relevance of the tetany index, as an indicator of the risk for sheep grazing pasture, is unclear. As forage K, Mg and Ca can impact the Mg and Ca status of lactating ewes, the calculation of a similar ratio may be possible; although it is not clear whether the threshold of 2.2 will vary between species.

Non-dietary influences on magnesium activity and function

Herd (1966b) noted that grass tetany mainly occurs in Border Leicester x Merino ewes mated for prime lamb production, with the disease appearing to be less prevalent in Merino and Corriedale ewes bred for wool

production. The author noted that this may reflect both breed and management differences (Herd 1966b). While it is unclear what differences in management are referred to, prime lamb production systems may lamb earlier in the calendar year compared to wool production systems to maximise lamb sale weights (Court *et al.* 2010), and this may correspond to more grass dominant pastures which are more tetany-prone (Smith *et al.* 1999). Breed differences in milk production (Morgan *et al.* 2007; Kremer *et al.* 2010) could also affect Mg balance through loss of maternal Mg in milk, and breed differences in susceptibility to hypomagnesaemia have also been reported in the United Kingdom (McDonald *et al.* 2011).

In mixed pastures, a higher proportion of clover can reduce the risk of grass tetany in cattle (Harris *et al.* 1983; Smith *et al.* 1999). The implication is that livestock may be at greater risk of hypomagnesaemia early in the season when clover content of pastures is low, or in situations where sheep are grazing grass monocultures such as dual-purpose wheat crops.

Short fasts may play an important role in precipitating grass tetany symptoms in susceptible animals (Herd 1966a). Change in diet or stress associated with being moved may also induce metabolic disease (Blumer *et al.* 1939; Schuster *et al.* 1969). Management to avoid stress, such as yarding of sheep, and consideration of ewe diet prior to grazing tetany-prone pastures may therefore be important considerations to prevent clinical hypomagnesaemia.

Conclusion

High lamb mortality remains a significant economic and ethical issue within the Australian sheep industry. Reports of an association between low Ca and Mg status and uterine inertia, prolapse, brain damage from hypoxia and lower lamb survival indicate that either an inadequate or unbalanced supply of Mg and Ca during late pregnancy may cause an increase in lamb mortality. Furthermore, Mg supplementation has been shown to improve the vitality of offspring and increase the concentration of immunoglobulins in colostrum, also potentially improving lamb survival. It is well known that some pastures grazed by lambing ewes are likely to be deficient in calcium and/or magnesium. However, there is limited evidence concerning whether sub-clinical deficiencies will reduce lamb survival. Further research is required to determine any impact of sub-clinical deficiencies on lamb survival and related biological processes, and whether provision of supplements can improve lamb survival cost-effectively.

3. PROJECT OBJECTIVES

- a) Determine to what extent the calcium and magnesium status of ewes is reduced during late pregnancy and early lactation when grazing common forages.
- b) Determine if reduced calcium and/or magnesium status in the ewe is associated with dystocia and/or increased lamb mortality.
- c) If required (based on the outcomes of objectives 1 and 2), assess the options for cost-effective manipulation of the calcium and magnesium status of reproducing ewes, and the effects on the incidence and consequences of sub-clinical hypocalcaemia and hypomagnesaemia.

4. SUCCESS IN ACHIEVING OBJECTIVES

All project objectives were successfully achieved. Measurements from 15 properties across NSW, SA, VIC and WA in 2016 demonstrated that while the soil and forage samples did not indicate a widespread risk of hypocalcaemia and hypomagnesaemia on commonly grazed forages, blood and urine samples indicated a significant number of animals were at risk of calcium and magnesium deficiency. This is supported by a further study at Tullamore in 2017 which found 45% of ewes had below adequate plasma calcium concentration (<90 mg/L) in late pregnancy, and a study at Wagga in 2018 where the percentage of ewes with adequate plasma magnesium concentration declined from 78% to 28% during late pregnancy, and the proportion with adequate plasma calcium levels declined from 76% to 19%. These data indicate that blood calcium and magnesium levels decline markedly in many flocks during late pregnancy to sub-optimal levels when grazing a range of common pastures.

The grazing studies conducted have not indicated an increased risk of dystocia with low calcium or magnesium levels, although it may be that insufficient numbers of ewes were used to detect small differences in ewe death rates, or that the level of sub-clinical deficiency was not sufficient to result in dystocia. Lamb mortality at a flock level varied widely and was not clearly associated with calcium and magnesium status, suggesting that variation in breed, environmental conditions at lambing and other management factors may have a larger impact where deficiencies are sub-clinical. The variability of management used in commercial enterprises makes it difficult to clearly identify relationships with mineral status, and two supplement studies were conducted to specifically test for beneficial impacts on ewe and lamb survival.

Replicated studies using six commercial properties showed no improvement in ewe or lamb survival, nor any reduction on the incidence of sub-clinical hypocalcaemia and hypomagnesaemia due to Ca and Mg supplementation. Negligible supplement intake on three of the six properties means this issue needs to be overcome for widespread adoption. In contrast, when pen-fed a pellet ration, where intake of supplement could be ensured as indicated by an increase in calcium and magnesium levels, ewes showed improved energy balance regulation. This could be expected to reduce the risk of hypocalcaemia, hypomagnesaemia and ketosis (pregnancy toxaemia), particularly under conditions where nutrition is restricted, as often occurs in grazing situations. Furthermore, in the controlled feeding experiment, contraction length and parturition length was numerically much (less than half) shorter for second-born lambs from ewes supplemented with Ca and/or Mg, but due to limited numbers was not statistically significant ($P=0.08$ and 0.06 , respectively). This indicates supplementation may have benefits in reducing the risk of dystocia. Given the low cost of the supplement options tested (\$0.01 and \$0.02 per head per day for the standard and low DCAD supplements), they are cost-effective options likely to reduce the incidence and consequences of both clinical and sub-clinical hypocalcaemia and hypomagnesaemia.

5. STUDY 1. EVALUATING THE CALCIUM AND MAGNESIUM STATUS OF GRAZING EWES AND RELATIONSHIPS WITH DYSTOCIA AND LAMB MORTALITY

This study detailed below has been published in the journal *Animal Production Science* (Hocking Edwards *et al.* 2018).

Aim

The aim of this study was to evaluate the extent of calcium and magnesium deficiencies in a range of common pastures and the ewes grazing them, whether there was any relationship between mineral status and the level of dystocia and lamb mortality.

Methods

The study was conducted with co-operating producers on commercial farms during 2016 with approval from the Charles Sturt University Animal Care and Ethics Committee (Approval number A16029). Farms were located across sheep-growing regions of Australia to allow for regional variability, and included farms in southern New South Wales (5), South Australia (5), Western Australia (4) and Victoria (2). Mainly twin-bearing flocks were used, which were either naturally mated or artificially inseminated. The farms are detailed in Table 5.1. Most farms used Merino ewes lambing in winter months.

Sampling

Ewes which had been ultrasound scanned with known fetal number, or for which lambing records were to be obtained, were used. The ewes were placed on lambing paddocks before or at the start of the experimental period, where the first sampling date commenced at approximately 139 to 148 days after the start of the joining period/insemination date. Most flocks of ewes had already been grazing their lambing paddock at this time, although some, due to scarce pasture, were not placed in their lambing paddock until the day of first sampling. For those flocks, the intention was for ewes to be grazing a similar pasture type as the lambing paddock. After the first sampling the ewes remained in their lambing paddocks until approximately two weeks after the completion of lambing, after which the final sampling and measurements were recorded.

At the start and end of the experimental period, 50 ewes within the flock were eartagged, body condition scored (Jeffries 1961) and blood and urine was collected from a random sample of these. Blood was collected using vacutainers, and urine was collected using the nasal occlusion method, from the same 10-15 ewes both at the start and end of the lambing period. At the end of lambing, udder examination of the 50 eartagged ewes was used to classify rearing status. The number of lambs present at the completion of the lambing period was recorded and used to calculate survival, and weight recorded for a random selection of 50 lambs. The number of

ewe deaths during the experimental period was also recorded, although it was not possible to obtain accurate cause of death for these ewes.

The type of pasture/forage grazed on each farm was recorded, including main species, legume content and live and dead content. Forage was collected as grab samples from the paddocks grazed on three occasions: at 30 days prior to the first blood sampling; 1-2 weeks prior to lambing; and at the end of lambing. The samples were collected from at least 10 equidistant sites located along a transect across each paddock. Collection was according to the instructions provided by the analytical laboratory (<http://csbp-fertilisers.com.au/csbp-lab>). Feed on offer was measured at the same times, using quadrats cut to ground level. Soil samples (0-10 cm) were also collected at these times according to the instructions provided by the analytical laboratory (<http://csbp-fertilisers.com.au/csbp-lab>).

Details on any supplementary feeding were also recorded (period fed, type of feed, estimated quantity, and any mineral supplements).

Sample processing and analysis

Forage samples were dried at 60° C and soil samples dried at 40° C prior to delivery to a commercial analytical laboratory (CSBP Soil and Plant Analysis Laboratory, Altona St, Bibra Lake, WA, 6163). 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 (CaCl₂), 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 or a FG302/312 portable refractometer, Australian Instrument Services, Melbourne). Sub-samples of plasma and urine were then placed on ice for transport before being frozen and then stored 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 2678 using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)) and creatinine (Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, Nathan Cobb Drive, Charles Sturt University, Wagga Wagga, NSW 2678, Creatinine BLOSRx78 kit), by the kinetic colour Jaffe method using Beckman Coulter AU480 analyser (Beckman Coulter Ltd, UK). 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).

Statistical analysis

Descriptive statistics were used. A Pearson correlation matrix (SAS software v6.1) was used to associate mineral status of pasture and urine concentrations. Blood and urine data for Hamilton was not available.

Results

Pasture on offer

FOO measured 1-2 weeks prior to lambing ranged from 702 to 3251 kg DM/ha and at the end of lambing ranged from 474 to 3604 kg DM/ha.

Ewe and lamb survival

The survival of lambs between flocks ranged from 67 to 93% (Table 5.2). While there was no ewe mortality in five flocks, the highest ewe mortality was 8%. The condition score of ewes 1-2 weeks prior to lambing ranged from 2.6 to 3.6 and at the end of lambing ranged from 2.2 to 3.3.

Pasture mineral composition

Mineral concentration in the pasture approximately 1 week before the start of lambing are closest to the time of lambing so are presented in this report, as the correlation with pastures collected at 30 days pre-lambing were similar. The number of farms with pasture minerals outside accepted limits was low (Table 5.3). A small proportion of farms had pasture Ca (1/16), Na (3/16) or S (1/16) below published requirements. Some farms (4/16) also had K above the maximum tolerable level. Pasture from all farms had a DCAD at a level that could increase the risk of hypocalcaemia, but only 2 had an elevated risk of hypomagnesaemia and none of the forage collected had an abnormal K/Na+Mg ratio.

Soil mineral analysis

The relationship between soil and the status of mineral in pasture and ewes was examined using a correlation matrix. There was a strong, significant positive relationship between Ca, Mg and K in soils ($P < 0.05$) but very little correlation between soil minerals and pasture minerals. The only significant relationships were between Exchangeable soil Na and pasture Mg ($r = 0.58$, $P < 0.05$) and pasture K ($r = -0.55$, $P < 0.05$). There was also a significant relationship between exchangeable Na on the soil and Na in urine ($r = 0.62$, $P < 0.05$).

Table 5.1. Description of farms used in 2016.

Farm no.	Location	State	Ewe no.	Lamb breed	Fetus no.	Start date	End date	Pasture type
1	Wagga Wagga	NSW	58	Merino	2	25 May	22 Jun	Lucerne/clover
2	Old Junee	NSW	82	Merino	1 or 2	11 Jul	1 Sep	annual ryegrass + oats
3	Old Junee	NSW	75	Merino	1 or 2	25 Jul	5 Sep	subclover, annual grass
4	Holbrook	NSW	51	Merino	2	9 Aug	12 Oct	annual grasses and phalaris, sub clover and broadleaf weeds
5	Cowra	NSW	53	Merino	2	20 Jun	14 Jul	Clover, phalaris, cocksfoot & annual grass (72.4;18.3;8.5;0.8)
6	Avenue Range	SA	280	Merino	2	9 Jun	17 Aug	mixed annuals
7	Penola	SA	200	Composite	2	21 Jun	15 Aug	phalaris and annual grasses
8	Minnipa	SA	50	Merino	2	20 Jun	31 Aug	mixed annuals (medic and annual grass)
9	Kingston	SA	263	Merino	2	25 Aug	28 Oct	mixed annuals
10	Robe	SA	205	Merino	2	24 Aug	24 Oct	phalaris
11	Harrow	VIC	126	Merino	2	18 Aug	12 Sep	phalaris based
12	Broomehill	WA	91	Merino	2	9 Jun	29 Jun	clover and annual grasses
13	Wagin	WA	75	Dohne	2	10 Jun	19 Jul	clover and annual grasses
14	Katanning	WA	51	Merino	1 or 2	20 Jun	25 Jul	n/a
15	Kendenup	WA	84	Merino	1 or 2	4 Jul	10 Aug	capeweed dominant and clover
16	Hamilton	VIC	54	White Suffolk	1 or 2	30 Aug	12 Sep	Phalaris, rye, clover, capeweed, barley grass

Table 5.2. Sheep and pasture production on 16 farms in 2016.

Farm no	Location	Lamb breed	Lamb survival (%)	Ewe mortality (%)	Feed on offer - start (kg DM/ha)	Feed on offer - end (kg DM/ha)	Condition score - start	Condition score - end
1	Wagga Wagga	Merino	75.0	5.17	1319	1462	2.6	2.6
2	Old Junee	Merino	72.9	2.44	889	1623	3.0	3.2
3	Old Junee	Merino	72.9	0.00	788	966	3.0	2.8
4	Holbrook	Merino	66.7	7.84 ¹	3251	3634	2.9	2.8
5	Cowra	Merino	80.4	0.00	1205	1255	2.8	2.8
6	Avenue Range	Merino	74.5	1.07	967	474	2.7	2.2
7	Penola	Composite	79.6	2.50	2100	944	3.4	3.0
8	Minnipa	Merino	78.6	2.00	1076	3509	3.7	3.4
9	Kingston	Merino	72.8	2.66	702	957	2.8	2.6
10	Robe	Merino	66.8	1.95	898	1053	3.0	2.9
11	Harrow	Merino	65.5	4.76	942	1586	3.2	2.9
12	Broomehill	Merino	68.7	3.30	1994	1413	3.0	2.8
13	Wagin	Dohne	80.0	0.00	1478	1611	3.3	3.3
14	Katanning	Merino	92.5	0.00	1869	1387	3.2	2.8
15	Kendenup	Merino	na ²	0.00	2246	2540	3.6	2.9
16	Hamilton	White Suffolk	81.6	0.00	1039	1123	3.2	3.1

¹ 3 confirmed deaths, 5 ewes missing.

² Inaccuracies were detected in the scanning results from this farm. Lamb survival results are incorrect and not presented.

Blood and urine analysis

Ewes on all farms had alkaline urine (pH>7.0) and on most farms high plasma K (12/15) and low plasma Na (14/15). There was no indication from the group means of plasma Ca that ewes were at risk of hypocalcaemia. However, on one third of farms 20% of ewes fell below the recommended level. Urine analysis also indicated that ewes from 14 of the 15 farms were in the marginal range for Ca status, and on all farms more than 20% of the sampled ewes had low Ca levels (Table 5.4). The mean plasma and urine Mg concentrations on all farms indicated a low risk of hypomagnesaemia. However, 20% of the sampled ewes on 6/15 farms had low plasma Mg concentrations.

Table 5.3. Concentration of major minerals and indicators of the risk of metabolic imbalance in pre-lambing pastures.

Farm no	Location	Ca (% DM)	Mg (% DM)	P (% DM)	K (% DM)	Na (% DM)	S (% DM)	Cl (% DM)	DCAD (meq/100g DM)	Tetany index	K/(Na+Mg) ratio	Total N (% 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	Wagga Wagga	1.03	0.20	0.50	3.51	0.02	0.38	0.19	61.7	1.3	5.2	6.2
2	Old Junee	0.52	0.17	0.31	3.21	0.05	0.29	0.25	59.2	2.1	5.1	4.5
3	Old Junee	0.68	0.22	0.38	2.99	0.07	0.27	0.35	52.9	1.5	3.6	5.1
4	Holbrook	0.56	0.24	0.38	1.94	0.43	0.35	0.31	37.8	1.0	1.3	4.7
5	Cowra	0.46	0.23	0.57	5.29	0.14	0.40	1.20	82.7	3.2	5.4	6.0
6	Avenue Range	0.98	0.23	0.39	2.49	0.79	0.26	1.39	42.7	0.9	1.2	3.2
7	Penola	0.43	0.22	0.41	2.66	0.51	0.27	1.37	34.8	1.7	1.7	3.4
8	Minnipa	0.42	0.14	0.20	2.68	0.64	0.16	1.58	41.9	2.1	1.7	2.0
9	Kingston	0.61	0.21	0.53	2.50	0.30	0.30	1.22	23.9	1.3	2.1	3.9
10	Robe	0.70	0.38	0.31	2.04	0.31	0.22	1.15	19.6	0.8	1.2	2.6
11	Harrow	0.42	0.17	0.32	2.77	0.22	0.27	0.96	36.6	2.0	3.0	3.1
12	Broomehill	0.97	0.34	0.32	1.54	0.48	0.22	0.90	21.2	0.5	0.8	2.9
13	Wagin	1.03	0.35	0.27	2.04	0.67	0.30	1.28	26.6	0.7	0.9	4.6
14	Katanning	0.99	0.35	0.21	1.32	0.89	0.21	1.16	26.7	0.4	0.5	2.7
15	Kendenup	0.99	0.32	0.31	2.47	0.49	0.24	1.40	30.1	0.8	1.3	3.3
16	Hamilton	0.38	0.23	0.47	3.45	0.53	0.33	1.40	51.3	2.3	2.1	4.5
Number outside limits		1	0	0	4	3	1	0	16	2	0	1

¹ Derived from Freer *et al.* (2007) and/or National Research Council (2005). ² Estimated from Takagi and Block (1991), 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 5.4. Blood and urine metabolites in samples collected 1- 2 weeks pre-lambing in 2016.

Farm no	Location	pH urine	Plasma Ca (mg/l)	Plasma K (mg/l)	Plasma Mg (mg/l)	Plasma Na (mg/l)	Urine Ca (μmol/mosmol)	Urine Mg (μmol/mosmol)	Urine Na (μmol/mosmol)
Required level		<7 ¹	>90 ²	>94/<195 ²	>18 ²	>3220 ²	>1 ³	>1 ⁴	>1 ⁵
1	Wagga Wagga	8.3	95.3	198.6	22.7	2902	0.39	10.1	1.4
2	Old Junee	8.5	98.7	195.6	23.1	3131	0.73	5.3	6.1
3	Old Junee	8.3	98.5	202.9	19.7	2852	0.49	3.5	0.8
4	Holbrook	8.4	108.6	210.9	22.9	3414	0.79	8.3	0.8
5	Cowra	8.2	104.5	201.5	25.7	2534	0.54	9.1	53.6
6	Avenue Range	8.0	102.1	207.5	24.8	2962	0.61	10.4	96.5
7	Penola	7.9	95.7	195.9	21.3	3055	0.19	5.2	96.6
8	Minnipa	7.8	104.2	196.5	18.2	2627	0.99	5.5	81.6
9	Kingston	8.0	113.2	212.4	19.8	2925	0.17	5.3	87.3
10	Robe	7.7	97.2	189.4	18.5	2830	0.46	4.7	127.5
11	Harrow	8.5	98.9	196.5	20.5	2934	0.18	1.9	68.6
12	Broomehill	8.3	91.4	194.0	20.8	2888	0.25	10.4	136.6
13	Wagin	8.1	98.6	215.4	21.8	2917	0.91	13.0	181.6
14	Katanning	7.8	86.4	196.4	20.7	2776	1.01	18.4	485.7
15	Kendenup	7.9	108.2	222.6	20.3	2976	0.51	7.1	190.1
No. outside limits		16	1	12	0	14	14	0	2
No. farms with >20% ewes outside limits		15	5	15	6	14	15	0	4

⁴¹ 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).

Fractional excretion of Ca, Mg, K and P are shown in Table 5.5. There are no published values indicative of deficiency but Ca fractional excretion on 10 of the 15 farms was below the range of 0.47 – 0.57% reported for sheep consuming a marginal Ca and Mg and high K diet (Bhanugopan *et al.* 2015).

Table 5.5. Fractional excretion of minerals in urine.

Farm no	Location	Ca fractional excretion (%)	K fractional excretion (%)	Mg fractional excretion (%)	Na fractional excretion (%)	P fractional excretion (%)
Required level						
1	Wagga Wagga	0.32	96.5	13.3	0.01	4.13
2	Old Junee	0.75	128.8	11.2	0.08	0.24
3	Old Junee	0.13	32.5	3.8	0.00	1.07
4	Holbrook	0.35	82.0	4.6	0.01	0.12
5	Cowra	0.19	66.5	6.4	0.57	0.26
6	Avenue Range	0.55	38.6	20.4	1.43	1.30
7	Penola	0.14	56.9	8.9	1.16	1.52
8	Minnipa	0.33	20.8	6.3	0.61	1.65
9	Kingston	0.10	31.5	11.5	1.15	3.30
10	Robe	0.26	38.5	10.1	1.69	1.53
11	Harrow	0.10	33.5	2.9	0.70	1.11
12	Broomehill	0.24	22.8	27.6	2.64	1.16
13	Wagin	0.78	54.6	32.0	3.17	0.72
14	Katanning	0.83	116.1	40.3	7.19	0.76
15	Kendenup	0.57	69.5	19.8	3.84	1.29
Number outside limits		10 ¹	na	na	na	na

¹ No published limits Bhanugopan *et al.* (2015) reported fractional excretion of 0.47 – 0.57% in sheep fed marginal levels of Ca and Mg with high K.

There was a significant negative association ($r=-0.57$) between Ca in urine and P in pasture one week pre-lambing (Table 5.6). There was also a positive association between Mg in urine and pasture levels of Ca, Mg and Na. Initial analyses suggested there was a trend ($P<0.1$) towards a positive association between Ca in urine and lamb survival, and between Mg in urine and lamb survival ($P<0.05$; $R^2=0.46$). There were also non-significant negative trends between Ca and Mg in urine and ewe mortality ($P>0.1$) and a positive trend between urine pH and ewe mortality ($P<0.1$). These relationships were heavily driven by results from a couple of sites, and removal of the data from these sites resulted in the relationships being much weaker and not significant, so the data is not convincing that these relationships exist, even though there is some biological justification. Given the expected effect of breed on lamb survival, and an inability to account for differences in lambing time, weather and other management factors which are known to influence survival, it is recommended that more controlled studies be undertaken to determine if such relationships exist.

Table 5.6. Pearson correlation coefficients between mineral concentrations in pastures 30 days (below diagonal) and 7 days (above diagonal) before the start of lambing and pre-lambing urine. Italicised values on the diagonal are correlation coefficients between pasture at 30 days and 7 days before the start of lambing. Significant ($P<0.05$) correlations are shown in bold.

		Pasture					Urine pre-lambing			
		Ca	Mg	K	Na	P	Ca	Mg	K	Na
Pasture	Ca	0.76	0.64	-0.41	0.37	-0.16	0.17	0.68	-0.22	0.47
pre-lambing	Mg	0.55	0.79	-0.53	0.41	-0.29	0.12	0.53	-0.13	0.63
	K	-0.27	-0.13	0.27	-0.63	0.66	-0.17	-0.30	0.25	-0.53
	Na	0.73	0.46	-0.43	0.68	-0.55	0.47	0.60	-0.17	0.72
	P	-0.12	0.04	0.74	-0.14	0.48	-0.57	-0.19	0.16	-0.51
Urine	Ca	0.14	0.06	-0.06	0.29	-0.39		0.53	0.42	0.36
pre-lambing	Mg	0.60	0.39	-0.14	0.53	-0.06			0.26	0.70
	K	-0.35	-0.22	0.67	-0.31	0.44				-0.03
	Na	0.36	0.43	-0.54	0.71	-0.44				

Discussion

There is no consistent or strong evidence from the analysis of forages that a Ca or Mg deficiency should be expected in the pregnant ewes monitored in the present study. Calcium and Mg concentrations in the pastures were above the requirement of 0.4% and 0.09% (Freer et al. 2007) on all but one farm. On most farms, Mg in forage was well above requirements.

Even with adequate Ca and Mg in pastures, an imbalance in other minerals may precipitate a deficiency of these two minerals. In particular, low Na together with high K will depress Mg availability (Suttle 2010) and low Mg status is a predisposing factor for hypocalcaemia (Herd 1965). There is evidence that such an imbalance increases the risk of hypocalcaemia and hypomagnesaemia in pregnant ewes grazing vegetative cereals (Masters *et al.* 2017). However, in the current study, Na in forage was usually above requirements and K was above the maximum tolerable level (National Research Council 2005) on 4 of the 15 farms studied. In comparison, 65% of grazed young cereal crops have K above the maximum tolerable level and Na below requirement (Masters *et al.* 2017). The risk of hypocalcaemia is also increased by the consumption of a high DCAD diet. Low-DCAD diets trigger the homeostatic mechanism of bone mobilisation and increase intestinal absorption of Ca to maintain plasma Ca (Block 1984; Scott *et al.* 1993; Wilkens *et al.* 2016). While the pastures analysed in the present study had a DCAD above the level associated with bone Ca mobilisation, they were still below those reported for grazing crops (Masters *et al.* 2017). The pasture analysis alone does not indicate a high risk of hypocalcaemia or hypomagnesaemia in pregnant ewes grazing winter pastures.

Analysis of plasma and urine are not entirely consistent with the expectations from pasture analysis. Urine pH was above 7 on all properties; a high urine pH is consistent with an increased risk of hypocalcaemia (Grant *et al.* 1992) and 87.5% of properties had a mean Ca in urine below the adequate concentration of 1 mmol/mosmol (Paynter 1996). The mean Ca concentration in plasma was below adequate on only one farm but a third of the farms had more than 20% of sampled ewes with below-adequate Ca in plasma. Calcium and pH of urine were similar to those observed in ewes grazing cereal crops (Masters *et al.* 2017) and indicated a risk of low-Ca status. Conversely, all measures on Mg were consistent with adequate Mg status at the flock level; however, 40% of the farms had more than 20% of sampled ewes with below the required concentration of plasma Mg. Indeed, as actual lambing date of individual ewes was not always known, the data may have underestimated the risk as some of the ewes sampled may not have been in late pregnancy. In conclusion, the analysis of plasma and urine indicated a possible risk of low or marginal Ca status but adequate Mg in the majority of flocks assessed. The presence of ewes with low Ca or Mg in the majority of flocks surveyed in late pregnancy indicated that a further investigation into a possible relationship between low mineral status and lamb mortality is justified. In addition, the potential link between condition score, pasture availability and animal mineral concentration requires further analysis.

The interpretation of pasture, urine and plasma analysis is based around published standard values that are associated with deficiency or adequacy of Ca and Mg under steady-state conditions. Pregnancy and lactation are not steady-state and sudden increases in requirements disrupt the homeostatic equilibrium. Sudden changes in requirements or disruptions to availability of these key minerals are usually the cause of hypocalcaemia and hypomagnesaemia (Freer *et al.* 2007; Suttle 2010). Therefore, while the analysis above provided no compelling evidence of likely hypocalcaemia and hypomagnesaemia, it did not exclude the possibility. The mineral concentrations in pre-lambing blood and urine samples suggested that a significant number of animals grazing southern Australian pastures during winter may be at risk of sub-clinical hypocalcaemia and hypomagnesaemia. Further work is required to establish whether this contributes to lamb mortality and, if so, whether appropriately designed mineral supplementation can improve lamb survival in southern Australian pasture systems.

6. STUDY 2. MINERAL SUPPLEMENTATION OF PEN-FED EWES TO IMPROVE IMMUNITY AND METABOLISM

Aim

The literature review suggested that the calcium and magnesium status of ewes has the potential to alter the metabolic health, energy metabolism and immune status of ewes, and deficiencies may increase the duration of parturition, which has been associated with a reduction in lamb survival. The aim of this study was to evaluate the effects of calcium and/or magnesium deficiency on calcium and magnesium metabolism, the incidence of hypoxia and acidemia, and duration of parturition.

Methods

Experimental design and management

Forty four Merino ewes (3-5 years), joined to a Merino ram and scanned as twin-bearing, with a mean live weight of 60.7 ± 0.3 kg and mean body condition score of 2.6 ± 0.3 were allocated for this study. Following a 10 to 14 days adaptation and habituation period in the pens with barley grain and wheaten hay, ewes were gradually transitioned to the custom-made pellets (made from barley, lupins, oat hulls, bentonite, millmix and canola). Ewes were randomly allocated into four treatment groups, Ca group (pellets with high Ca), Mg group (pellets with high Mg), Ca/Mg group (pellet with high Ca and high Mg) and control group (pellets with adequate Ca and Mg). There were N=11 ewes in each treatment group (Table 6.1). The animals were housed in individual pens and fed individually throughout the experimental period.

The duration of the experiment was from 4 weeks pre-lambing to 4 weeks post lambing. The animals were fed their respective experimental diets daily throughout the duration of the experiment. The ewes were fed 2.5 kg DM of pellet/head/day (ME= 12 MJ/Kg DM, CP= 14.3%) during gestation and this was increased to 3 kg DM of pellets/head/day during lactation. Any refusals were measured and recorded each day in order to determine intake.

Sampling from ewes

The live weight of ewes was recorded at the start and at the end of the trial (4 weeks after lambing). Body condition score (BCS) of ewes were recorded each week.

Blood through jugular venepuncture and urine samples through the nasal occlusion method were collected at

the start of the experiment and then weekly from ewes pre lambing. Post lambing blood and urine samples were collected within 12 to 24 hours and then every 2 weeks until 4 weeks post lambing. The blood samples were centrifuged, plasma separated and were stored in -20°C for further analysis of minerals, antioxidants and hormones.

Colostrum samples were collected within 12 – 24 hours of lambing from the ewes and stored at -20°C for further analysis of minerals and IgG. Milk samples were collected every 2 weeks until 4 weeks after lambing and stored at -20°C for further analysis of minerals.

Table 6.1. Proximate and mineral analysis of feed on offer for ewes during late gestation and early lactation.

	Treatment			
	Control	Calcium	Magnesium	Calcium + Magnesium
Dry Matter (%)	90.4	90.4	90.4	90.4
NDF (%)	33.6	33.6	33.6	33.6
ADF (%)	15.8	15.8	15.8	15.8
Crude protein (%)	14.4	14.4	14.4	14.4
ME (MJ/kg DM)	12.2	12.2	12.2	12.2
Crude fibre (%)	8.2	8.2	8.2	8.2
Calcium (%)	0.33	0.71	0.33	0.66
Magnesium (%)	0.28	0.29	0.43	0.47
Potassium (%)	0.72	0.73	0.79	0.81
Sodium(%)	0.20	0.21	0.20	0.23
Phosphorus (%)	0.36	0.34	0.43	0.43

NDF – neutral detergent fibre; ADF – acid detergent fibre; ME – metabolisable energy

Sampling from lambs

The live weight of lambs was recorded at birth, and then at two weekly intervals up until 4 weeks after lambing. Blood samples were collected by jugular venepuncture within 12 to 24 hours after birth and at 2 weekly intervals up until 4 weeks (inclusive) at the end of the experiment. The plasma was separated and stored at -20°C for further analysis.

Ewe and lamb behaviour

A continuous video record was made using 12 CCTV cameras (NVC-DFI, Dome camera) and 24 infrared lights (140 LED illuminator light 80m) during the last three weeks of gestation. Duration of labour for the first lamb was calculated from the first visible contraction to the full expulsion of the lamb (Labour 1) (Dwyer *et al.* 1996).

The number and duration of contractions for delivery of both lambs were recorded. As the ewes were twin bearing ewes, the duration of labour for the second lamb was regarded as the duration from full expulsion of the first lamb until full expulsion of the second lamb (Labour 2). Continuous recording of each ewe was used to record the occurrence and duration of isolation, laying down, standing up and walking. All changes in posture between standing and laying and all bouts of urination and pawing were also counted in ewes.

Continuous sampling from lambs was used to record duration of grooming, latency to stand and walk, and the number of attempts to stand and suckle for each lamb (Martin *et al.* 2007). Following analysis of the video footage, a lamb vigour score was given for each lamb (Hergenhahn *et al.* 2014).

Feed samples

Throughout the experimental period, pellets were sampled weekly. All weekly samples of pellets were bulked, mixed thoroughly and a homogenised 0.5 kg sample stored to be sent to the Department of Primary Industry (DPI), Wagga Wagga, NSW, Australia for proximate and mineral analysis.

Statistical analysis

All data were analysed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corporation. Distributions of the residuals of continuous data were evaluated for normality by using frequency histograms and Q-Q plots. All data were analysed by linear mixed models. Both the main effects (treatment, timepoint, rearing status), and their interaction effects (timepoint×treatment, treatment×rearing status and timepoint×treatment×rearing status) were considered as fixed factors. Ewe and pen were included as random factors. For the variables TAC, ROM, OSi, plasma Ca, plasma Mg, urine Ca: creatinine and urine Mg: creatinine of ewes, the effect of difference between treatment groups at the starting time point was included as a covariate in the statistical model. A P-value of < 0.05 was used to identify differences that were statistically significant and $P \geq 0.05$ but ≤ 0.1 are used to show the tendencies. The data collected from lambs that died during the experimental period were excluded from the data analysis.

Results

The concentration of calcium, magnesium and phosphorus in plasma, milk and urine in ewes

Plasma Ca concentration in ewes increased from one week prior to the commencement of lambing to the end of study ($P<0.001$); however, treatment did not have a significant effect on plasma Ca concentrations ($P=0.178$). The interaction of time \times treatment was not significant ($P=0.966$) for plasma Ca concentration. Plasma Mg concentration increased over time ($P<0.001$) and ewes supplemented with Mg (Mg and Ca+Mg groups) had greater plasma Mg concentration throughout the experimental period compared to the control and the Ca group ($P<0.001$). The interaction of time \times treatment was significant and plasma Mg was higher in Mg and Ca+Mg groups at +4 weeks compared to the other time points ($P=0.002$) (Figure 6.1). Plasma P increased over time ($P<0.001$) but treatment had no significant effect on the plasma P concentration ($P=0.672$). The interaction of time \times treatment was not significant for plasma P concentration ($P=0.156$).

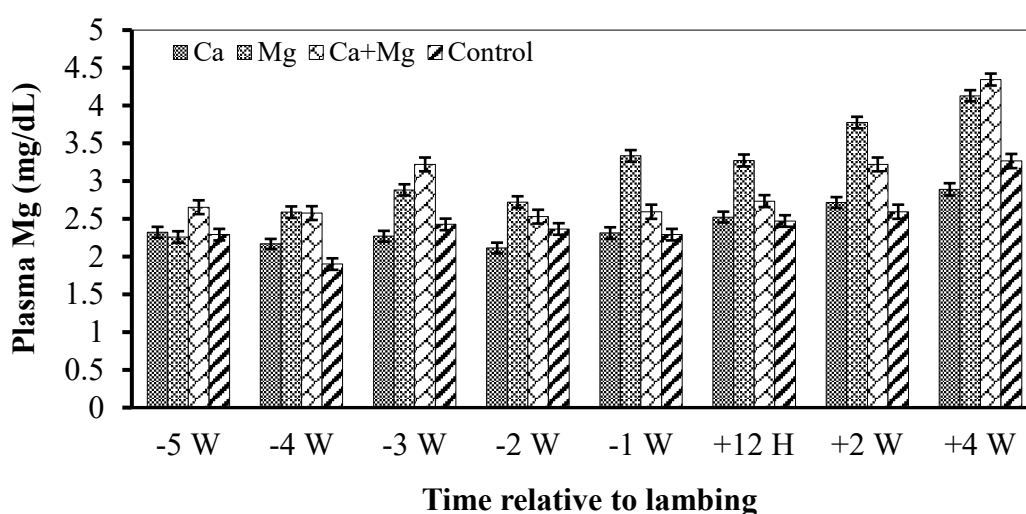


Figure 6.1. Plasma magnesium concentration in ewes fed different levels of calcium and magnesium from one month before lambing to one month post lambing (W=week, H=hour, bars are SEM).

The urinary Mg: Creatinine ratio was lowest and urinary Ca: Creatinine was highest at lambing ($P<0.001$). The urinary P: Creatinine ratio increased from lambing onwards ($P<0.001$). Treatment did not have a significant effect on urinary Ca: Creatinine ratio ($P=0.109$). However, Mg supplementation increased Mg excretion through urine ($P<0.001$), and P excretion through urine was higher in the control and Ca+Mg groups than the other groups ($P=0.001$) (Table 6.2).

The concentration of Ca, Mg and P in colostrum did not differ significantly between treatment groups ($P>0.308$). The Ca concentration in milk was significantly higher at week 4 compared to week 2 ($P=0.021$), while milk Mg and P concentration decreased over the same period ($P<0.001$). However, milk Ca, Mg and P concentration did not differ significantly between treatments at any time point ($P>0.115$).

Table 6.2. The effect of calcium and magnesium supplementation on urine calcium/creatinine ratio, urine magnesium/ creatinine ratio and urine phosphorus/ creatinine ratio.

Time	Treatment				P value		
	Control	Calcium	Magnesium	Calcium + Magnesium	Time	Treatment	Time× Treatment
Urine Calcium: Creatinine ratio							
-5 Weeks	4.2±0.20	3.3±0.19	2.4±0.21	3.5±0.24	0.00	0.109	0.00
-4 Weeks	1.6±0.19	3.9±0.17	2.8±0.24	2.6±0.24			
-3 Weeks	3.1±0.20	4.5±0.17	4.0±0.22	1.7±0.24			
-2 Weeks	3.0±0.20	1.5±0.17	1.8±0.20	1.8±0.24			
-1 Weeks	2.2±0.19	1.6±0.20	2.1±0.20	2.7±0.24			
+12 Hours	2.7±0.23	5.5±0.20	1.3±0.34	3.5±0.23			
+2 Weeks	1.9±0.24	2.2±0.19	1.5±0.20	1.8±0.24			
+4 Weeks	1.8±0.24	2.0±0.20	1.6±0.19	1.8±0.20			
Urine Magnesium: Creatinine ratio							
-5 Weeks	0.4±0.07	0.3±0.07	0.3±0.09	0.4±0.08	0.002	0.00	0.577
-4 Weeks	0.3±0.08	0.4±0.07	0.5±0.08	0.7±0.08			
-3 Weeks	0.3±0.08	0.3±0.07	0.7±0.08	0.5±0.07			
-2 Weeks	0.3±0.08	0.26±0.07	0.4±0.08	0.6±0.08			
-1 Weeks	0.2±0.08	0.3±0.08	0.5±0.08	0.5±0.08			
+12 Hours	0.1±0.08	0.2±0.08	0.2±0.10	0.3±0.08			
+2 Weeks	0.3±0.09	0.2±0.08	0.5±0.08	0.5±0.08			
+4 Weeks	0.4±0.09	0.3±0.08	0.5±0.08	0.6±0.08			
Urine Phosphorus: Creatinine ratio							
-5 Weeks	1.3±0.46	1.0±0.43	1.0±0.49	1.2±0.48	0.00	0.001	0.036
-4 Weeks	1.2±0.46	1.1±0.43	1.0±0.52	1.2±0.48			
-3 Weeks	1.1±0.49	1.0±0.41	1.0±0.52	1.2±0.45			
-2 Weeks	1.2±0.49	1.0±0.41	1.0±0.49	1.3±0.48			
-1 Weeks	2.8±0.46	1.3±0.48	1.3±0.46	1.3±0.48			
+12 Hours	27.3±0.49	13.2±0.48	31.0±0.64	26.2±0.52			
+2 Weeks	20.5±0.57	9.6±0.48	10.7±0.48	24.1±0.47			
+4 Weeks	9.5±.57	15.1±0.46	11.7±0.48	31.2±0.47			

Plasma PTH, 25 hydroxy vitamin D3 and 1,25 di hydroxy vitamin D3 concentrations

The concentration of hormones 25(OH)D₃, 1, 25(OH)₂D₃ and parathyroid hormone (PTH) are represented in Table 6.3. PTH and 1,25(OH)₂D₃ are hormones involved in calcium homeostasis or plasma Ca regulation. The mean plasma concentration of PTH, 1,25(OH)₂D₃ and 25(OH)D₃ was the highest at one week prior to lambing compared to the other time points ($P \leq 0.002$). Ewes from the Ca+Mg group had the lowest mean concentration of 1,25(OH)₂D₃ ($P=0.005$) compared to the other groups and the mean concentration of PTH in this group tended to be lower than the other groups ($P=0.06$).

Table 6.3. The effect of calcium and magnesium supplementation on the concentration of hormones PTH, 25 Hydroxy Vitamin D and 1, 25 dihydroxy Vitamin D in ewes.

	Treatment				P-value		
	Control	Ca	Mg	Ca+Mg	time	treat	time × treat
1,25(OH)2D3 (pmol/L)							
-5 Weeks	238.1±105.8	400.03±105.8	434.4±104.1	295.4±100.4	0.001	0.005	n.s.
-1 Weeks	233.7±110.5	338.7±105.8	510.1±100.4	279.4±100.4			
+12 Hours	123.2±106.3	328.1±110.3	448.3±106.7	115.6±106.7			
+2 Weeks	161.8±124.6	223.0±106.3	272.2±101.8	88.3±101.8			
25(OH)D3 (mmol/L)							
-5 Weeks	178.1±58.30	207.4±51.2	214.8±54.54	180.6±51.23	0.001	n.s.	n.s.
-1 Weeks	134.9±53.73	146.2±51.9	224.8±53.73	122.5±51.23			
+12 Hours	164.3±53.73	176.0±51.3	272.3±55.12	128.7±53.50			
+2 Weeks	171.6±53.73	90.5±53.9	204.5±54.04	75.9±53.50			
PTH (pg/ml)							
-5 Weeks	116.1±37.82	124.5±36.10	127.0±38.37	104.2±36.06	0.002	0.06	0.08
-1 Weeks	132.1±37.82	110.4±37.81	218.5±37.82	104.7±37.97			
+12 Hours	109.3±37.82	118.9±36.13	172.3±39.38	91.0±41.91			
+2 Weeks	110.0±42.9	95.1±37.94	136.1±38.17	41.0±39.2			

Mineral profile in lamb plasma

Plasma Ca and Mg in lambs decreased over time ($P<0.001$). Plasma Mg concentration in lambs from the supplemented groups was higher than the control group ($P=0.023$). There was a significant effect of time × treatment interaction on plasma Mg concentration in which lambs from the control group had lower plasma Mg concentration compared to the treatment groups at +4 W ($P=0.001$), but not at other sampling times (Figure 6.2). Plasma Ca level in lambs of the Ca and Ca+Mg groups had a trend to be higher than the control group ($P=0.082$), although the time × treatment interaction was not significant. Plasma P levels in lambs were highest at +2 W ($P<0.001$). Lambs in the control group had lower plasma P concentration compared to the other treatment groups ($P=0.037$).

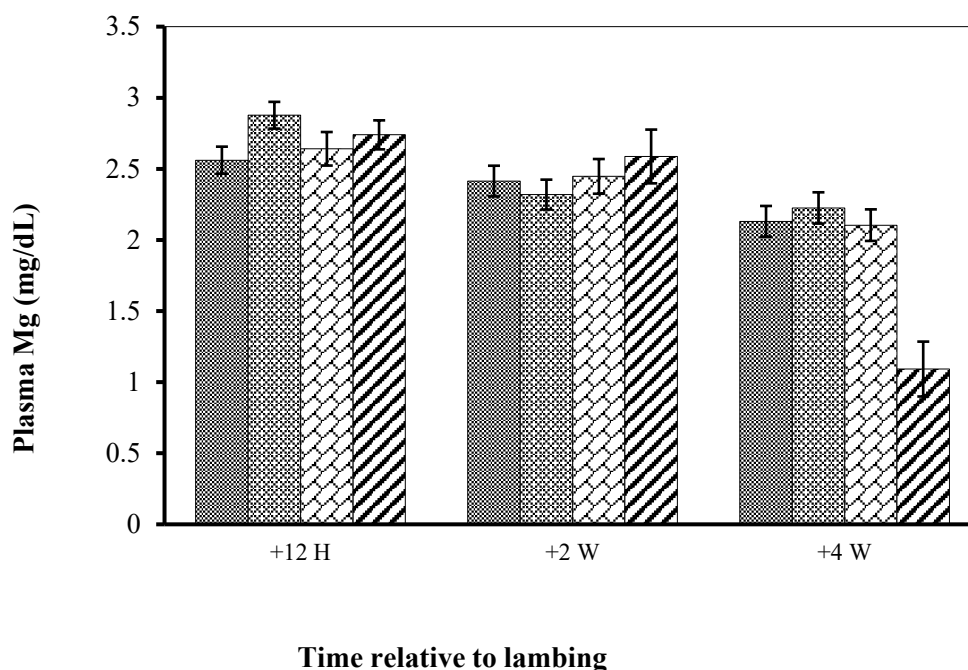






Figure 6.2. Changes in plasma magnesium concentration in lambs as a result of maternal supplementation with calcium and magnesium from one month prior to lambing to one month post lambing. (W" represents weeks, "H" represents "hours" and error bar represents SEM). Ca  Mg  Ca+Mg  Control 

Effect of maternal calcium and magnesium supplementation on immune factors in ewes and lambs

Oxidative status in ewes

The oxidative burst response of leukocytes in ewes improved from the beginning of lambing through to four weeks post lambing ($P < 0.001$). Treatment did not have a significant effect on the oxidative burst response of ewes ($P = 0.149$). In ewes, the phagocytosis function of leukocytes was highest at lambing compared to the other time points ($P < 0.001$), but no significant effect of treatment was observed on phagocytosis function of leukocytes ($P = 0.401$). Mean total plasma antioxidant capacity (TAC) ($\mu\text{mol HCl/ml}$) in ewes was lowest and mean reactive oxygen species (ROM) (Carr U) and OSi (ROM/TAC) were the greatest at lambing ($P < 0.001$), and there was no treatment effect on either TAC or ROM at any time point.

The concentration of non-esterified fatty acids (NEFA) was higher at lambing compared to the other time points ($P < 0.001$), and the control group had a higher NEFA level at lambing compared to the other groups at this time point ($P = 0.02$) (Figure 6.3). The body condition score of ewes decreased over time ($P = 0.037$) with ewes being in BCS 2.4 at 4 weeks post-lambing; however, treatment did not have a significant effect on BCS of ewes ($P = 0.811$).

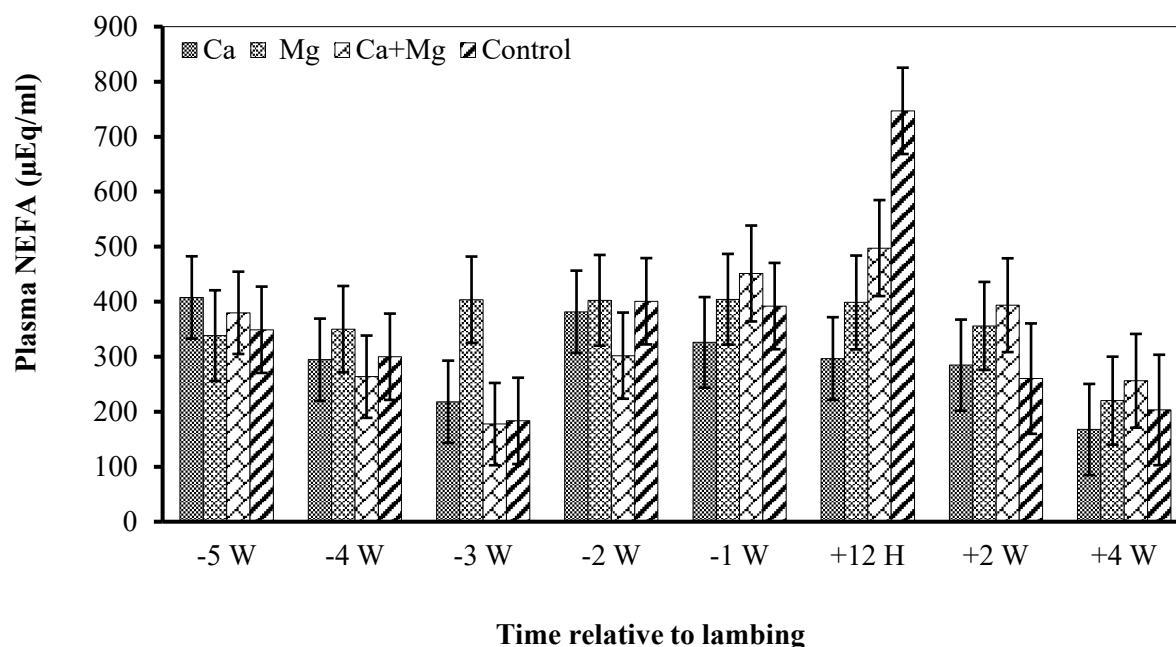


Figure 6.3. Mean plasma NEFA concentration ($\mu\text{Eq/ml}$) in ewes supplemented with calcium and magnesium from one month prior to lambing to one month post lambing.

Immune status of lambs

There was a significant increase in oxidative burst response and phagocytosis function of leukocytes in lambs over time ($P \leq 0.015$). Lambs in the Ca group had greater oxidative burst response compared to the other groups at +4 weeks (treatment \times time interaction $P = 0.051$) (Figure 6.4); however, phagocytosis function of leukocytes did not differ with treatment ($P = 0.450$).

Treatment did not have a significant effect on colostrum IgG concentration ($P = 0.250$). The concentration of IgG at +2 W ($20.1 \pm 1.48 \mu\text{g/ml}$) in lambs was higher than at lambing ($14.0 \pm 1.74 \mu\text{g/ml}$) and +4 weeks ($11.7 \pm 1.32 \mu\text{g/ml}$) ($P < 0.001$). The effect of treatment on plasma IgG concentration was not significant, but had a trend to be higher in Ca the group ($19.4 \pm 1.68 \mu\text{g/ml}$) compared to the Mg group ($14.7 \pm 1.98 \mu\text{g/ml}$), Ca+Mg group ($13.6 \pm 1.69 \mu\text{g/ml}$) and control group ($14.2 \pm 2.38 \mu\text{g/ml}$) ($P = 0.055$). The time \times treatment interaction tended

towards significance ($P=0.097$), with plasma IgG concentration being higher in lambs from the Ca group at +4 W.

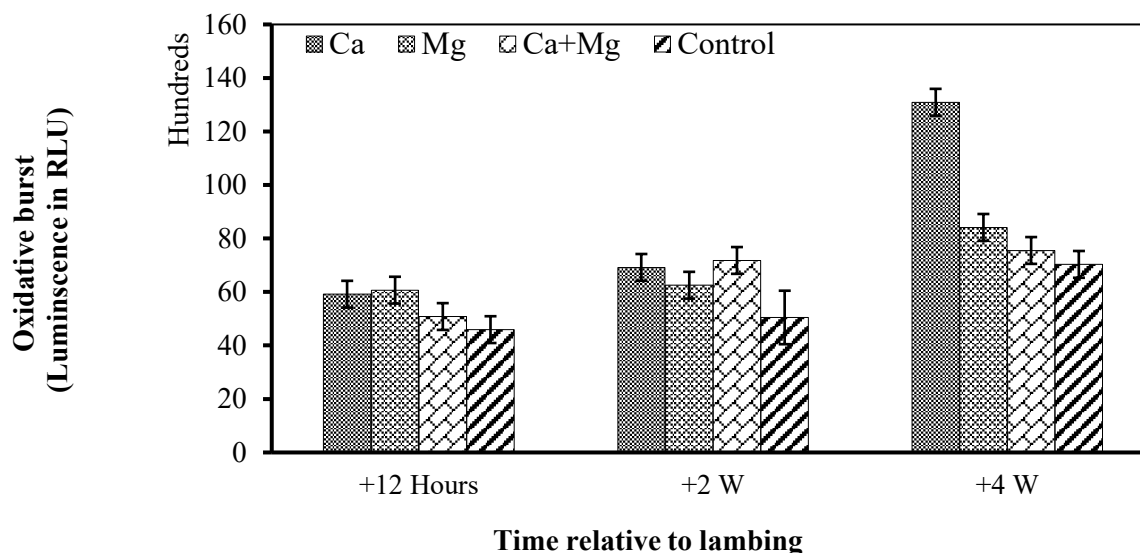


Figure 6.4. Oxidative burst response of leukocytes in lambs as a result of maternal calcium and magnesium supplementation from lambing to 4 weeks of age. W” represents weeks, “H” represents “hours” and error bar represents SE.

Total plasma antioxidant capacity (TAC) significantly decreased from +12 hours to +2 W ($P<0.001$) but did not change significantly from +2 W to +4 W. Lambs in the Mg (395.5 ± 20.40 $\mu\text{mol HCl/ml}$) and Ca+Mg (362.1 ± 19.49 $\mu\text{mol HCl/ml}$) groups had greater TAC compared to the Ca group (315.3 ± 16.57 $\mu\text{mol HCl/ml}$) and control groups (296.5 ± 24.30 $\mu\text{mol HCl/ml}$) ($P=0.04$). The mean concentration of reactive oxygen metabolites (ROM) in lamb plasma was higher at +2 W (262.8 ± 14.50 Carr U) than at lambing (53.9 ± 13.58 Carr U) and +4 weeks (147.3 ± 13.02 $\mu\text{mol Carr U}$), but there was no treatment effect. Oxidative stress index (OSI) in lambs was significantly greater at +2 W than at other times ($P<0.001$), and was lower in Mg supplemented groups at +2 W than the other groups ($P=0.031$).

Lamb live weight

The live weight of lambs over time increased significantly ($P<0.001$). There was a significant treatment \times time interaction for lamb weight ($P=0.008$; Figure 6.5). Lambs in the supplemented groups had greater weight gain from birth to 4 weeks of age compared to the lambs from the control group ($P=0.007$), with most of this benefit

being apparent in the period from 2 weeks of age to 4 weeks of age. The average daily weight gain of treatment groups (from birth to 4 weeks of age) was 204 g/head.day, 207 g/head.day, 245 g/head.day for Ca, Mg and Ca+Mg groups respectively, which was significantly higher than the control group (148 g/head.day) ($P<0.001$).

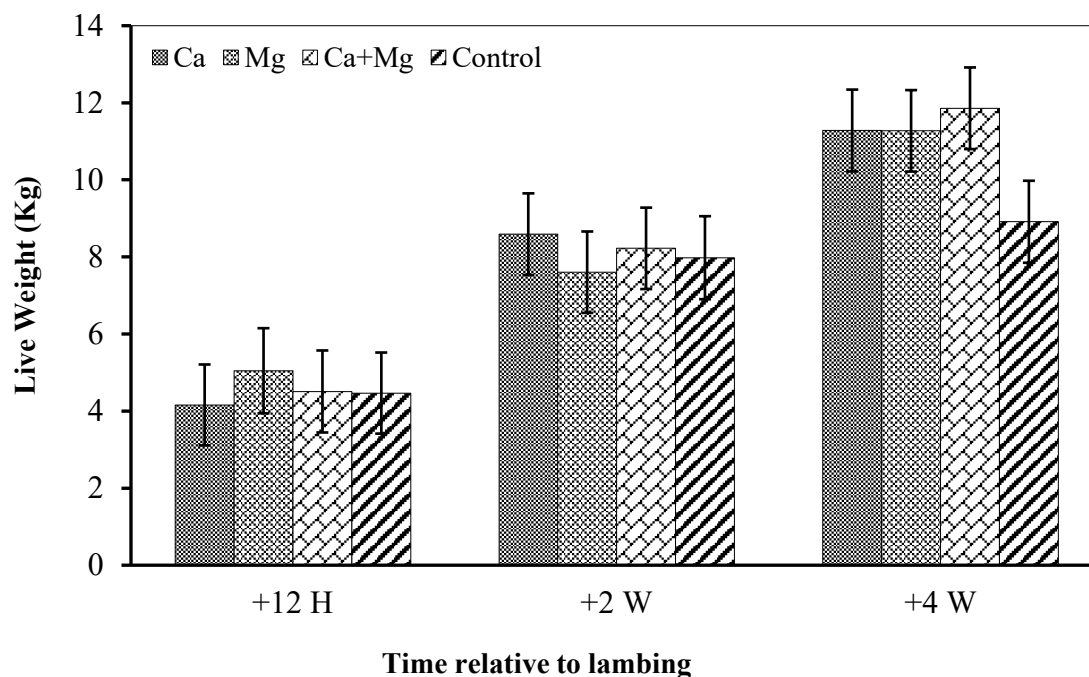


Figure 6.5. Live weight of lambs from lambing to 4 weeks of age. (“W” represents weeks, “H” represents “hours” and error bar represents SEM)

Behavioural observations

The contraction frequency, contraction duration and parturition duration of ewes for both lambs are presented in Table 6.4. The effect of treatment on variables such as contraction frequency and contraction duration for the first lamb was insignificant ($P \geq 0.117$). The effect of treatment on contraction duration ($P=0.075$) and parturition duration ($P=0.058$) for the second lamb had a trend toward being shorter in the supplemented groups compared to the control group. Treatment did not affect contraction numbers for the second lamb ($P=0.193$). There was no effect of treatment on lamb behaviour.

Discussion

The results of this study showed that supplementation of twin-bearing ewes with Ca, Mg or both during the last month of gestation through to the first month of lactation regulated the Ca homeostatic mechanism and improved the energy balance of ewes. Moreover, maternal supplementation boosted the immune response in

lambs as evidenced by an increase in the total antioxidant capacity and oxidative burst response of leukocyte cells, and also increased live weight of lambs at 4 weeks of age.

There was no incidence of metabolic diseases in any of the treatment groups throughout the study.

Supplementation of ewes with Mg improved plasma Mg concentration in ewes, because plasma Mg concentration is not under hormonal control but is dependent on dietary Mg levels and is regulated by ruminal absorption and kidney excretion.

Table 6.4. Effect of treatment on pre-parturition and parturient behaviours of ewes. N=number

Variables	Control	Ca	Mg	Ca+Mg	P value
Pre-parturient behaviours					
Standing up & laying down (N)	12.6±2.22	9.9±2.48	9.2±2.22	13.1±2.34	0.547
Aimless walking (N)	272.9±1.44	222.8±1.47	288.4±1.44	378.4±1.47	0.806
Urination frequency (N)	2.6±1.31	3.6±1.33	2.6±1.31	2.9±1.33	0.809
Parturient behaviours-Lamb 1					
Contraction frequency (N)	28.3±1.21	15.3±1.22	22.7±1.21	27.4±1.22	0.117
Contraction Length (seconds)	411.1±1.19	307.6±1.20	272.3±1.19	422.7±1.20	0.215
Parturition Length (minutes)	148.3±1.276	71.0±1.276	111.4±1.262	134.0±1.276	0.172
Parturient behaviours-Lamb 2					
Contraction frequency (N)	5.0±1.32	2.3±1.44	3.4±1.37	2.1±1.40	0.193
Contraction Length (seconds)	105.0±1.35	56.5±1.47	45.4±1.39	31.0±1.43	0.075
Parturition Length (minutes)	49.2±1.35	16.9±1.37	16.1±1.40	21.9±1.48	0.058

Supplementation with Ca did not improve plasma Ca levels due to homeostatic control of Ca. As demands for Ca are high in late gestation and early lactation, ewes try to cope with high Ca requirements through hormonal homeostasis (Braithwaite 1983). The plasma concentrations of these hormones (PTH and 1,25(OH)₂D₃) were high at all pre-lambing time points, and the lower plasma Ca concentrations at these time were in line with findings of Kadzere *et al.* (1997). A lower concentration of PTH and 1,25(OH)₂D₃ in the Ca+Mg group suggests that ewes in this group required a lower homeostatic response to maintain their plasma Ca concentrations. This phenomenon could be due to increased availability of Ca and Mg in the diet facilitating intestinal Ca absorption and decreasing the demand for Ca regulating mechanisms. These findings were further supported with high Ca excretion through the urine in the Ca+Mg group. Ewes from this group had higher Ca excretion through the urine compared to the other treatment groups and it is reported that urinary excretion of Ca can be used as an index to ascertain the Ca status of the animal (Bhanugopan *et al.* 2015; Masters *et al.* 2017); thus, higher urinary

Ca excretion, normal plasma Ca and lower concentration of hormones suggests higher Ca status in the Ca+Mg supplemented group.

Maternal supplementation from one month prior to lambing to one month post-lambing significantly boosted the immune response in lambs. The highest plasma concentration of TAC in lambs was at 12 hours after birth and it significantly decreased at 2 weeks of age suggesting that high mean TAC at birth was a result of passive immunity transferred by the colostrum. Improvement of mean TAC in lambs from maternal supplementation with Mg in this study (both the Mg and Ca+Mg treatments) could be associated with passive transfer of TAC through the consumption of colostrum, since Mg has a structural role in antioxidants such as glutathione peroxidase (GSH-PX) which is well known to be a superoxide radical scavenger (Nawito *et al.* 2016). Further studies are required to evaluate the level of TAC in colostrum and evaluate the effect of Mg supplementation on TAC concentration in colostrum. The results cannot be easily compared with the literature, since to the best of our knowledge this is the first study that has evaluated oxidative stress in neonatal lambs born from mothers supplemented with Ca and Mg. Mean ROM at the first few hours of life in lambs was low compared to 2 and 4 weeks of age which is in agreement with the study of Abuelo *et al.* (2014), in which calves showed higher mean ROM at first week after birth compared to the day of birth. It seems that the high concentration of TAC present at birth were capable of neutralizing the ROM generated at the same time in lambs of the current study, since antioxidants are the main elements that neutralize ROM (Albera & Kankofer 2011; Abuelo *et al.* 2015).

At 4 weeks of age, lambs from dams supplemented with Ca had greater oxidative burst than the other groups which could be the result of an improvement in the mobilization of intracellular Ca into the leukocyte's cytoplasm, and consequently resulting in efficient signal transduction which possibly sped up the process of leukocyte maturation. These findings were further supported by the higher IgG concentration in lambs from Ca supplemented groups at 4 weeks of age, which most probably is related to the effect of Ca in leukocyte maturation and accordingly higher IgG synthesis by B-cells. Future research should evaluate Ca concentration in mature and immature leukocytes to investigate the mechanisms involved in leukocyte maturation.

Supplementation with Ca and Mg improved the live weight of lambs at 4 weeks of age. The immunity status of lambs has been shown to increase the nutrient absorption from the intestine (Walkden-Brown & Kahn 2002) and consequently may affect lamb weight gain. Moreover, improved lamb immune status might have had an effect on the dam-lamb bond which influences milk consumption and lamb weight. Weight of lambs at around lamb marking is an important factor that governs weaning weight and time of weaning.

This study showed that Ca and Mg can improve regulation of energy metabolism. High energy demands of ewes at lambing were identified by high NEFA concentration in plasma. The improved energy regulation at lambing in ewes receiving mineral supplements was likely due to the role of Ca and Mg in glucose turnover, endogenous glucose production, regulation of insulin level, regulation of ATP production and muscle contraction. The latter

influences rumen and abomasum motility, digestive tract motility and feed intake of animals (Cinar *et al.* 2008; Martinez *et al.* 2012).

Supplementation of ewes with Ca and Mg in this study tended ($P < 0.10$) to reduce parturition and contraction length for the second lamb. While the effect was not statistically significant, the relatively small sample size, combined with the high variability of these traits and the large numerical difference (two-fold difference), strongly suggests that the effect would have been statistically significant had more animals been included in the study. A P value of 0.10 indicates 90% confidence that the difference is real and not random. Ca and Mg are important for efficient muscle contraction under hormonal influences, and the application of these hormones in the absence of extracellular Ca and ATP (as an energy source) causes a significant decrease in contraction amplitude and initiate only small contractions (Wray & Arrowsmith 2012b).

No significant difference between treatment groups was observed for the parturition duration of the first lamb, likely because the Ca and Mg concentration of the base pellet offered to the control group were sufficient to regulate energy for the delivery of the first lamb. As parturition is an energy consuming process, our findings suggest that ewes from the control group were not able to regulate energy as efficiently as the supplemented groups for the delivery of the second lamb. Better regulation of energy with Ca and Mg supplementation is also evidenced by the lower concentration of NEFA in plasma of supplemented ewes at lambing compared to control ewes. For ethical reasons in this study, we were not able to feed a diet deficient in Ca and Mg to the control group, but further studies could be undertaken to determine whether mineral supplementation influences parturition measures in deficient ewes. Nonetheless, the trend for contraction and parturition length to be shorter in the supplemented groups suggests that supplementation should be beneficial even when there is no deficiency.

7. STUDY 3. MINERAL SUPPLEMENTATION OF GRAZING EWES TO IMPROVE LAMB SURVIVAL

Aim

Both the literature and previous studies indicated that sub-clinical calcium and magnesium deficiencies are common in commercial flocks grazing pastures, with a potential to increase the rate of dystocia and reduce lamb survival. The pen study described in section 6 showed that mineral supplementation can improve the mineral and metabolic status of ewes, and the immune response in lambs, providing further evidence of potential for a positive impact of supplementation on lamb survival. The aim of the current study was to evaluate whether mineral supplementation of ewes grazing common pastures would increase lamb survival.

Methods

All procedures were conducted with the approval of the Charles Sturt University Animal Care and Ethics Committee (project A17030), and the study was conducted during 2017.

A randomised block design was used comprising 2 replicates of 2 treatments conducted on each of 5 properties. The treatments were an unsupplemented control, and a mineral supplementation treatment given to lambing ewes. The mineral supplement was formulated to provide a low cation-anion difference (DCAD), and was comprised of magnesium chloride, calcium sulphate, and salt ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}:\text{CaSO}_4 \cdot 2\text{H}_2\text{O}:\text{NaCl}$), in the ratio 12.5:32.5:55.0, fed at a rate of 30 g/ewe per day. An intake of 20g of supplement was estimated to provide 25% of Mg, 40% of Ca, and 487% of Na requirements.

Sheep management and measurements

Sites on five commercial properties were selected at Holbrook and Tullamore (NSW), Robe and Kingston (SA) and at Pingelly (WA). Merino ewes which had been joined to Merino rams and ultrasound scanned as twin-bearing were used. On each property, between 400 and 600 mature-age (not maidens) 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. Where possible, paddocks were subdivided to provide blocking for treatments. A description of each site is shown in Table 7.1.

Table 7.1. Description of experimental sites used in 2017.

	Tullamore	Pingelly	Kingston	Robe	Holbrook
State	NSW	WA	SA	SA	NSW
Lambing start	9 Jul	20 Jul	1 Sep	1 Sep	17 Aug
No. ewes/ location	450	400	400	400	600
Paddock size (ha)	60	15-18	21	22-30	10
Pasture	Barley grass/ clover	Capeweed/ subclover	Phalaris/annual grass/clover	Phalaris based	Phalaris/clover/ annual grass
Grass%	64	26	42	68	95

Between 6 and 10 days before the start of lambing, a random sample of 50 ewes per paddock were condition-scored (Jefferies 1961). Blood samples were collected from 10 ewes per paddock using 9 ml heparin vacutainers and stored on ice for transport. Blood was centrifuged and plasma frozen at -20°C until analysis. Urine samples were collected from the same 10 ewes by nasal occlusion. Urine pH was measured immediately using a pH meter (Pingelly - Thermo Scientific Orion Star A325; SA - TPS WP-80), and specific gravity measured using a refractometer (Tullamore, Pingelly and SA – clinical urine refractometer TE-RM200SGRI, Testequip; Holbrook - FG302/312 portable refractometer, Australian Instrument Services, Melbourne). Urine samples were then stored on ice for transport, and frozen -20°C until analysis. After sampling, each group of ewes was placed into a lambing paddock (10 to 60 ha), and mineral supplementation commenced. Sufficient resources across sites were available to only permit the collection of blood and urine at one sampling time – it was deemed more important to collect this pre-supplementation to determine baseline levels, given we knew from the pen study and other studies (e.g. Masters *et al.* in press) that supplementation can alter levels.

Minerals were fed throughout the lambing period in troughs, with troughs replenished twice weekly, and refusals collected once weekly and weighed to estimate intake. Where necessary, subsamples of refusals were weighed, dried, and re-weighed to calculate dry weights. A small quantity of pellets or grain was mixed with the minerals at some locations at the start of feeding to attract ewes, but was discontinued due to being ineffective. Ewes at Tullamore were fed oaten grain at 0.5 kg/ewe/day throughout the lambing period due to inadequate pasture, but ewes at other sites did not require supplementary feed.

Approximately two weeks after the end of lambing, each paddock of sheep was mustered, and the same 50 ewes condition scored. Lambs present were counted, and lamb survival calculated as lambs present at marking age/foetuses placed in paddock.

The quantity of live and dead pasture was measured from 10 quadrat cuts per paddock, both at the time of blood sampling pre-lambing, then also post-lambing. Cuts were sorted into components, dried at 60°C, then weighed.

Laboratory analyses

The concentration of Mg, Ca and P in plasma and urine samples was analysed using kits (photometric colour test method). Creatinine was analysed using a creatinine BLOSRx78 kit (kinetic colour Jaffe method) and a Beckman Coulter AU480 analyser (Beckman Coulter Ltd, UK). Fractional excretion of each mineral was calculated as $(\text{concentration in urine} \times \text{plasma concentration of creatinine}) / (\text{plasma concentration} \times \text{urine concentration of creatinine})$, converted to percentage (Bhanugopan *et al.* 2015). The concentrations of Ca, Mg and Na in urine were converted from mmol/L to $\mu\text{mol/mosmol}$ using the equation: $\text{osmolality (mosmol/L)} = -39231 + 39214 \times \text{specific gravity}$ (English and Hogan 1979).

Statistical analysis

Data for locations at Holbrook, Robe and Kingston were not analysed as little of the supplement was consumed, but means and sem are presented where available for completeness.

For Tullamore and Pingelly locations, where mineral intake was over 10 g/ewe per day, plot means for lamb survival, condition score and pasture biomass were analysed using linear mixed models with location x treatment as the fixed effect and location.rep as the random term. Plasma and urine data were analysed with similar models using individual ewe data, with the exceptions that urine pH was only available for the Pingelly location, and an exponential transformation was used for this variable prior to analysis. Urine Ca and Mg data was log transformed prior to analysis. Transformations were inadequate to equalise variances for plasma Ca and fractional excretions of P, so these data sets were analysed using the nonparametric Wilcoxin rank sum test.

Results

Mineral supplement intake

Supplement intake was below the target quantity of 30 g/ewe per day at all sites, and was negligible at Kingston, Robe and Holbrook (Table 7.2).

Lamb survival

The survival of lambs was higher ($P < 0.001$) at Tullamore ($80 \pm 1.6\%$) than at Pingelly ($59 \pm 1.6\%$) but was not increased ($P = 0.345$) by mineral supplementation. There was no interaction between location and treatment. Lamb survival varied at other locations (Fig. 7.1) but was also similar between treatments.

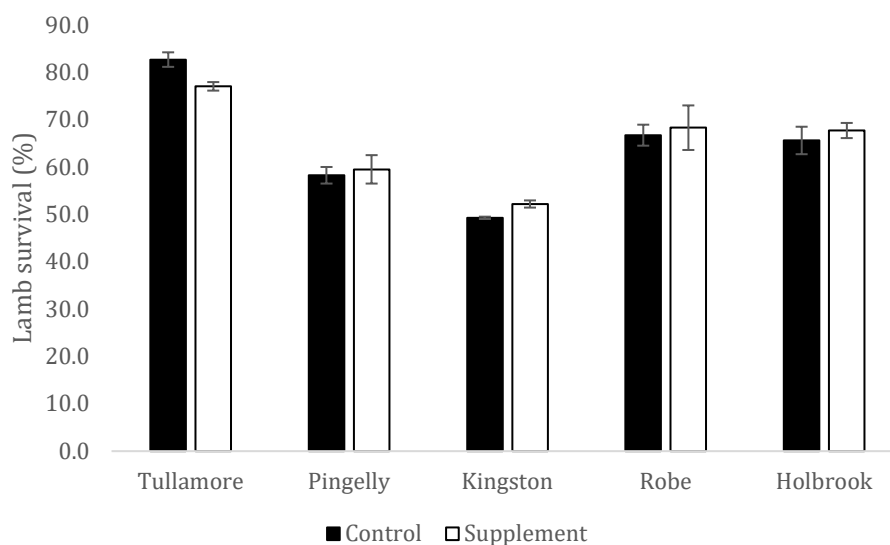


Figure 7.1. Mean lamb survival (%) of control and supplemented treatments at five locations in 2017. Values are raw means \pm sem.

Condition score

Ewe condition score was similar between treatments both pre and post-lambing. The mean condition score of ewes was higher ($P < 0.001$) at Tullamore (3.2 ± 0.023) than Pingelly (3.0 ± 0.023) pre-lambing, but post-lambing, ewe condition score was similar at both locations (2.9 ± 0.04). The condition score of ewes at all other locations was 3.0 to 3.3 pre-lambing, and 2.7 to 2.9 post-lambing.

Pasture availability and supplementary feed

The mean quantity of live pasture pre and post-lambing was similar between treatments. While pasture pre-lambing was similar between the Tullamore and Pingelly locations (444 ± 65) kg DM/ha, post-lambing Pingelly provided a larger quantity (1359 ± 103 kg DM/ha vs 301 ± 103 kg DM/ha). The quantity of live pasture at other locations was above 1300 kg DM/ha pre lambing, and above 1500 kg DM/ha post-lambing (Table 7.2).

Supplementary feeding with 0.5 kg/ewe per day of oat grain was required at Tullamore throughout the lambing period due to the low quantity of live pasture. Ewes at Pingelly and the other locations were not supplementary fed.

Table 7.2. Mean supplement intake and live pasture availability at five locations (raw means).

	Tullamore	Pingelly	Kingston	Robe	Holbrook
Supplement intake (g/ewe/day) ^A	19.0	12.6	0.4	2.02	8.05
Live pasture pre-lambing (kg DM/ha)	455	432	1868	1308	2830
Live pasture post-lambing (kg DM/ha)	301	1359	3033	3069	1690

^ASupplemented groups only.

Plasma and urine variables

Prior to mineral supplementation and lambing, for the locations analysed there were few differences in mineral concentrations between control and supplemented ewes, nor between locations (Table 7.3).

Mg in urine was higher in the supplemented than control ewes, but in plasma was similar between treatments. Fractional excretion of calcium showed an interaction between location and treatment, due to relatively higher levels in control ewes at Tullamore. Raw means for pre-lambing urine pH and specific gravity for locations where data was not statistically analysed are shown in Table 7.4.

The percentage of ewes with mineral levels in the adequate range is shown in Table 7.5. While most ewes at both Tullamore and Pingelly showed adequate plasma concentrations of Mg, only 55% of Pingelly ewes had adequate (>90 mg/L) calcium levels. However, only 10% of the Pingelly ewes showed Ca plasma levels of 80 mg/L or less, indicating that the degree of deficiency was not severe. While P levels were adequate at Pingelly, at Tullamore most ewes were deficient.

Table 7.3. Urine and plasma variables pre-lambing for control and mineral supplemented ewes at Tullamore and Pingelly in 2017.

		Tullamore	Pingelly	Trt	P values Location	Trt x Location
Urine pH ^A	Control	-	7.56	0.562	-	-
	Supplement	-	7.31		-	-
Ca plasma (mg/L) ^B	Control	97.12 (97.4)	88.16 (89)	0.673	0.004	-
	Supplement	93.60 (93.8)	92.44 (93.2)			
Ca urine ^C (μ mol/mosmol)	Control	0.030	0.007	0.183	0.173	0.141
	Supplement	0.029	0.015			
Mg plasma (mg/L)	Control	20.75	21.38	0.062	0.773	0.118
	Supplement	23.38	21.63			
Mg urine ^C (μ mol/mosmol)	Control	0.212	0.123	0.007	0.203	0.288
	Supplement	0.387	0.162			
P plasma (mg/L)	Control	32.89	58.71	0.445	0.109	0.653
	Supplement	31.59	53.72			
P urine (μ mol/mosmol)	Control	0.031	0.013	0.208	0.529	0.146
	Supplement	0.011	0.014			
FE Ca (%)	Control	1.1614*	0.5974	0.226	0.294	0.009
	Supplement	0.6646	0.7755			
FE Mg (%)	Control	26.21	21.83	0.078	0.574	0.355
	Supplement	21.32	20.27			
FE P (%) ^B	Control	0.44 (0.03)	0.08 (0)	0.956	0.864	-
	Supplement	0.28 (0)	0.09 (0.06)			

Cre = creatinine, Ca = calcium, Mg = magnesium, P=phosphorus, FE = fractional excretion.

*One outlying data point removed

^ARaw means; data transformed before analysis

^BRaw means, data for treatment and location analysed by non-parametric test; median values in brackets.

^CBacktransformed means

Table 7.4. Raw mean urine pH and specific gravity pre-lambing for control and mineral supplemented ewes at Kingston, Robe and Holbrook in 2017.

	Treatment	Kingston	Robe	Holbrook
Urine pH ^A	Control	8.18	7.49	8.20
	Supplement	8.19	7.67	8.00
Specific gravity	Control	1.012	1.016	1.017
	Supplement	1.013	1.011	1.004

Table 5. Percentage of ewes with mineral levels within the adequate range.

	Tullamore	Pingelly
Ca plasma > 90 mg/L	85	55
Mg plasma > 18 mg/L	100	90
P plasma > 45 mg/L	15	90
Ca urine < 1 μ mol/mosmol	100	100
Mg urine < 1 μ mol/mosmol	100	100

Discussion

Mineral supplementation did not increase lamb survival at the two locations where above 10 g/ewe/day supplement was consumed. The lack of response may have been due to pre-lambing plasma/urine levels of Mg and Ca being adequate in the majority of ewes at Tullamore prior to mineral supplementation. At Pingelly, Ca was only adequate in over half of the ewes, but no ewes were reported with clinical Ca deficiency, and most ewe had Mg levels in the adequate range.

It is not clear what percentage of ewes would need to be deficient in calcium or magnesium, nor the severity of sub-clinical deficiency, in order for supplementation with minerals to improve lamb survival. Furthermore, since the intake of supplement at Pingelly was low, it is unknown whether sufficient intake was obtained to achieve a response in survival. The large percentage of ewes at Tullamore which were deficient in P does not lend evidence to this mineral having a large influence on perinatal lamb survival. The level of lamb survival of 80% is

at the higher end of reports for twin-born lambs (Hinch & Brien 2014), so it is likely that mineral supplementation alone could improve this further.

A response in lamb survival might not be expected if Ca and Mg levels were already adequate. While previous studies have shown Ca and Mg deficiencies occur on grass-dominant pastures, both the Tullamore and Pingelly sites used pastures with a subclover or broadleaf component. These are considered representative of many commercial pastures, since grass-only pastures, other than grazing cereals, are not recommended due to reduced weight gain of sheep in comparison with pastures containing a legume component, and the benefits to nitrogen fixation from the legume component. The results suggest that mineral supplementation may not increase lamb survival on mixed pastures where ewes are not showing clinical deficiency.

It is not clear whether higher than adequate mineral concentrations have the potential to improve lamb survival. The lack of response may have been due to insufficient mineral intake as intake was below the target at all locations. Whether the low intake was driven by the type of supplement used or some other factor is unclear, although a very similar supplement was readily consumed by sheep grazing cereal crops (Masters *et al.* in press). In future studies both pre and post- supplementation blood and urine sampling is advisable to establish whether an increase in mineral levels, and to what level, is associated with any production response.

The mineral supplement used in this study was not readily consumed on several sites, with low levels of intake possibly exacerbated by high quantities of live pasture at most locations. Tullamore, the only location with near-target mineral intake, was the only site with very low quantities of live pasture throughout. The level of consumption needs to be improved in future studies, or the situations identified where adequate intake can be achieved.

It is concluded that mineral supplementation that achieved moderate supplement intake (12-19g/hd/day) did not increase perinatal lamb survival in ewes grazing mixed pastures when most ewes had adequate Ca and Mg levels. Strategies that promote higher supplement intake may be more effective, especially in flocks where a significant proportion of ewes have marginal Ca and/or Mg status.

8. STUDY 4. MINERAL SUPPLEMENTATION OF GRAZING EWES TO IMPROVE SURVIVAL AND MINERAL STATUS

Aim

This study repeated the key component of the 2017 supplement trials, with the objective of achieving adequate supplement intake, which did not occur on several of the 2017 sites. Given it was unclear whether the type of supplement (low DCAD) used in 2017 may have been less palatable, this study included a standard supplement that is commonly used and known to be palatable. The aim of this study was to determine whether supplementation of lambing ewes grazing pasture would improve mineral status and increase the level of lamb survival while also reducing the incidence of sub-clinical or clinical metabolic disorders.

Methods

All procedures were conducted with the approval of the Charles Sturt University Animal Care and Ethics Committee, and the study was conducted during 2018 on the CSU commercial farm at Wagga Wagga (NSW).

Experimental design

The study was designed with two replicates of three treatments:

Control – no mineral supplement

Standard – a loose mix of lime, causmag and salt in the ratio 1:1:1. It was considered that this mix, commonly used in industry, might be more palatable than the Low DCAD treatment

Low DCAD - a low cation-anion difference (DCAD), comprised of magnesium chloride, calcium sulphate and salt ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O} : \text{CaSO}_4 \cdot 2\text{H}_2\text{O} : \text{NaCl}$) in the ratio 12.5:32.5:55.0, as used in the 2017 study.

Adjacent paddocks containing lucerne (*Medicago sativa*) pasture were subdivided to create six plots, but with incomplete blocking due to the size and location of paddocks. All plots were between 19 and 23.5 ha in size.

Sheep management and measurements

Mature composite ewes joined to composite rams and due to lamb from 6 July were scanned for fetal number, and 600 twin-bearing ewes selected. On day 120 from the start of joining, 90 ewes were randomly selected and blood samples collected by venupuncture into 9ml heparin vacutainers. The blood was stored on ice before being centrifuged and plasma frozen at -20°C until analysis. Urine samples were collected from the same ewes by nasal occlusion. Urine pH was measured using a pH meter, and frozen at -20°C until analysis. The following day (7 June 2018) the ewes were moved to the experimental site and randomly divided into 6 groups (n=100/group), ensuring 15 sampled ewes per group. A random sample of 50 ewes per group were condition-scored (Jefferies 1961) before each group was randomly allocated to a lambing plot. The groups were re-yarded on day 140 (26 June) and the same ewes re-sampled. Due to the low quantity of available pasture, ewes were supplementary fed cereal grain (wheat and barley) at 0.8 to 1.5 kg/ewe/day while on lambing plots.

Mineral supplementation commenced on 9 June, 120 days after joining commenced and approximately one month prior to the start of lambing. The supplements were fed in troughs, at a rate of 30g/ewe/day until 22 June, after which it was reduced to 20 g/ewe/day (based on observed intake prior) until 3 August when it was estimated that 90% of ewes had lambed. The minerals were fed every one to two days for the first week of feeding, after which they were fed every 3 to 4 days. Refusals were collected, dried if necessary and weighed to calculate intake.

When the youngest lambs were a week old (13 August), the sheep in each plot were yarded separately. Lambs surviving to this (marking) age were counted and weighed, and lamb survival calculated as lambs present at marking age per fetus placed in the plot. The condition score of ewes was also recorded at this time. Records were kept of ewe mortality for each plot throughout the lambing period, although cause of death was not usually diagnosed.

The quantity of live pasture was visually estimated in 100 quadrats (0.1m²) per plot. These estimates were calibrated against 20 quadrats which were estimated, cut with electric clippers, dried at 60°C, then weighed, using the method of (Haydock & Shaw 1975).

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 2678). Creatinine was analysed using a creatinine BLOSRx78 kit (kinetic colour Jaffe method) and a Beckman Coulter AU480 analyser (Beckman Coulter Ltd, UK). Fractional excretion of each mineral was calculated as (concentration in urine x plasma concentration of creatinine)/(plasma concentration x urine concentration of creatinine), converted to percentage (Bhanugopan *et al.* 2015).

Statistical analysis

Data was 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 x treatment as the fixed effect and plot or plot + plot.ewe as the random term. Urine data was transformed by logarithm prior to analysis, with one outlier removed from the calcium data. The weight of lambs, percentage lamb survival and quantity of pasture were also analysed using linear mixed models, with treatment as the fixed and plot as the random term.

Results

Mineral supplement intake

Supplement intake was calculated as 18.2 g/ewe/day for the standard and 19.6 g/ewe/day for the low DCAD supplements. The ewes readily consumed both supplements, with refusals indicating intake was *ad libitum* much of the time.

Production variables

Production data is shown in Table 8.1. The quantity of live pasture available pre-lambing was similar between treatments, as was lamb survival and the mean weight of lambs. Mean ewe condition score was similar between treatments ($P=0.131$) and although it was higher ($P<0.001$) at day 120 (3.1) than at day 140 of pregnancy (3.0) or at the completion of lambing (3.0), this difference is not considered of practical importance.

Table 8.1. Pasture available and lamb production from three mineral supplementation treatments.

	Control	Low DCAD	Standard	P value
Live pasture available (kg DM/ha)	554 \pm 333	423 \pm 333	751 \pm 333	0.796
Lamb survival (%)	77 \pm 3.8	77.3 \pm 3.8	77.0 \pm 3.8	0.999
Mean lamb weight (kg)	12.65	12.89	13.65	0.539

Blood and urine mineral concentrations are shown in Table 8.2. Mean calcium levels in plasma declined between day 120 and 140 from the start of joining, but there were no differences or interactions between treatments. Most ewes in each treatment showed adequate calcium levels (>90 mg/L) at day 120, but less than 25% were adequate by day 140, in all treatments. Plasma magnesium levels in the low DCAD treatment were higher than the control and standard at day 120, but there were no differences between treatments at day 140. The proportion of ewes with adequate magnesium levels (>18 mg/L) declined during late pregnancy and was less than 30% in all treatments at day 140.

Urinary calcium levels did not change over time and were similar between treatments. For urinary magnesium, while there were no differences between treatments at either sampling time, levels increased between day 120 and 140 in the control, but remained stable in the supplemented treatments. Urine pH increased during late pregnancy, but was similar between treatments.

Urinary fractional excretion of calcium was similar between treatments but was higher at day 140 than 120 (Table 8.3). Fractional excretion of Mg was higher at day 140, but while neither supplement differed from the control, excretion was lower in DCAD than the standard supplement. Supplementation with either supplement increased excretion of Na at day 140 compared with the control, although all treatments were similar at day 120 before supplementation.

Table 8.2. Plasma and urine calcium (Ca), magnesium (Mg) and sodium (Na) concentrations and urine pH from three mineral supplementation treatments on days 120 and 140 from joining at Wagga Wagga in 2018.

	Day	Control	Low DCAD	Standard	P value trt	P value day	P value trt x day
Plasma calcium (mg/L)	120	92.9	101.1	95.4	0.261	<0.001	0.192
	140	83.5	82.5	83.9			
Proportion of ewes with Ca>90 mg/L	120	0.73	0.82	0.73	0.628	<0.001	0.817
	140	0.14	0.23	0.23			
Plasma magnesium (mg/L)	120	19.5	23.4	21.1	0.028	<0.001	0.021
	140	17.2	16.9	17.0			
Proportion of ewes with Mg>18 mg/L	120	0.62	0.91	0.72	0.511	<0.001	0.143
	140	0.28	0.27	0.28			
Urine Ca mg/L ^A	120	42.2	32.5	32.6	0.934	0.94	0.241
	140	50.0	26.2	27.9			
Urine Mg mg/L ^A	120	124	269	144	0.779	<0.001	0.041
	140	255	162	246			
Urine Na mg/L ^A	120	4.8	17.6	5.6	0.092	<0.001	0.002
	140	89.6	423.0	221.6			
Urine pH	120	6.7	6.9	6.8	0.447	<0.001	0.197
	140	8.2	7.8	8.1			

^ARaw means – data log transformed for analysis.

Ewe health

A total of 14 ewes died during the study. The mortality rate was similar between treatments (2% in control and low DCAD, 3% in the standard). The cause of death for most ewes was unclear, although two were lambing complications. Only one ewe (control) was treated for hypocalcaemia, but was unresponsive.

Table 8.3. Urinary fractional excretion of calcium (Ca), magnesium (Mg) and sodium (Na) from three mineral supplementation treatments on days 120 and 140 from joining at Wagga Wagga in 2018.

	Day	Control	Low DCAD	Standard	P value trt	P value day	P value trt x day
Calcium (%) ^A	120	0.26	0.21	0.22	0.668	0.024	0.777
	140	0.56	0.32	0.44			
Magnesium (%)	120	5.32	4.53	4.55	0.238	<0.001	<0.001
	140	14.87	11.79	21.43			
Sodium (%) ^A	120	0.003	0.004	0.002	0.006	<0.001	0.005
	140	0.013	0.227	0.064			

^AData was square root transformed, back-transformed means are shown.

Discussion

Mineral supplementation did not improve lamb survival, and did not appear to alter the rate of dystocia nor clinical metabolic disease. Despite supplement consumption at near the target level, the calcium and magnesium concentrations in plasma were largely unaltered compared with unsupplemented ewes, and the majority of ewes in all treatments showed deficient levels by day 140 after joining. This is in contrast to previous studies (e.g. pen study in this project, Masters *et al.* in press) where supplementation with magnesium increased plasma Mg levels in both ewes and lambs. The low DCAD diet was effective, however, at reducing urinary Mg

concentration and fractional excretion of Mg in comparison to the other treatments. This could be expected to put ewes in an improved metabolic state.

Plasma Ca and Mg levels at day 120 in this experiment were within the range of values observed in the 2016 survey, but at day 140 the values were at the lower end observed in the survey. In particular, plasma Mg levels at d140 in this experiment were lower than those observed in both the intensive pen feeding experiment and the 2017 flock supplementation trial. Dietary mineral supplementation in this experiment did not raise plasma levels to a level sufficient to remove the majority of ewes out of the risk zone – with less than 25% of ewes having plasma Ca levels above 90mg/L, and less than 30% of ewes having plasma Mg above 18mg/L. It is likely that this precluded any response in mechanisms that lead to improved lamb survival, such as the improved ewe energy balance and lamb immune function as observed in the intensive pen study. The high level of grain supplementation in this experiment, due to adverse seasonal conditions, may have been the cause for so many ewes being marginal in Ca and Mg status in late pregnancy. Masters *et al.* (in press) observed higher plasma Ca levels in unsupplemented ewes grazing cereal crops than those observed in the present study, and supplementation with either a standard or low DCAD supplement was effective at increasing plasma Ca in many of these flocks. Plasma Mg levels in the present study were also at the lower end of the range observed by Masters *et al.* (in press), and supplementation improved plasma Mg levels in the flock with similar levels to that observed in the present study. Supplement intakes in the study of Masters *et al.* (in press) were higher (up to 30g/hd/d) in many flocks compared to the present study, but not in the flock with similar plasma Mg levels where supplementation was effective. Clearly interactions between herbage availability and type, any grain supplementation, and the existing metabolic status of the ewes will affect not only supplement intake and the metabolic response of the ewes. Age of the ewes and perhaps even breed (the previous studies used mostly Merinos or Merino cross) may also influence the response. Further work is required before firm recommendations can be given as to the likely response to supplementation with Ca and Mg under a range of grazing conditions.

9. GENERAL DISCUSSION

The comprehensive review of the literature found evidence indicative of potential mechanisms whereby sub-clinical calcium and magnesium deficiencies might reduce lamb survival. Such mechanisms are likely to impact on lamb survival through increased incidence of dystocia, and also potentially through reducing newborn lamb viability through reductions in immunity. The effect of Ca and Mg on these mechanisms was confirmed through an intensive pen feeding study, whereby it was demonstrated that increasing the Ca and/or Mg content of the diet (above 'adequate' levels, as per feeding standards) improved the energy regulation of late pregnant ewes (potentially reducing the incidence of ketosis and improving ewe vigour at lambing), and also the immune status of newborn lambs (potentially improving newborn viability). There was also a trend ($P < 0.1$) for a reduction in parturition duration of second-born lambs from supplemented twin bearing ewes, which if repeatable could be expected to improve the viability of second-born lambs. Supplementation with Ca and/or Mg also increased the weight gain of lambs to 4 weeks of age. Lamb survival was not improved in this study, although given the small number of animals included the study was not designed to test this.

One third of the 16 flocks sampled across southern Australia during 2016 had more than 20% of ewes with below adequate calcium or magnesium a week before lambing, when grazing typical pastures. Given the importance of Ca and Mg in mechanisms potentially affecting lamb survival identified in the review and demonstrated in the pen study, this provided a compelling case to investigate whether supplementation of Ca and Mg at the flock level could improve lamb survival. Low intake of the supplement at three of the five sites tested in 2017 reduced the ability to test the efficacy of supplementation, but at the two sites where supplement was consumed in amounts above 10g/ewe daily, there was no effect on lamb survival. A further experiment in 2018 compared two forms of supplementation providing Ca and Mg – a low DCAD form and a 'standard' form (lime, Causmag and salt) and observed similar intakes (20g/hd/day) of each, but no effect of supplementation on plasma or urinary Ca or Mg levels, and no effect on lamb survival.

It is concluded that many late-pregnant ewes in Australian grazing flocks are sub-clinically deficient in calcium and magnesium. While the supplementation studies undertaken on commercial flocks grazing common pastures did not show any improvement in lamb survival, this may have been due to variable supplement intake between animals limiting any flock level response in lamb survival. Alternatively, given increased dietary intake of Ca and Mg were shown to improve mechanisms (ewe energy status, newborn immunity) that could be expected to improve lamb survival, it is possible that increasing supply of Ca and Mg in the commercial flocks did not result in

improvements in lamb survival because the energy balance of the ewes and/or lamb immunity were not compromised in these flocks. It is also possible, especially for the 2018 study, that the level of supplement intake of near 20g/d was insufficient to improve the Ca and Mg status of ewes to a level which could result in improvements in mechanisms leading to improved survival, given the high proportion of sampled ewes which were marginal in Ca and Mg status. This does not preclude supplementation being effective in other flocks.

Supplementation of Ca and Mg can be cost minimal. For example, the raw components used in the standard supplement cost 1c/hd/day, while the low DCAD supplement costs 2c/hd/day when offered at 30g/hd/day. Labour and other costs associated with mixing and feeding these supplements out would need to be costed at the individual farm level, but feeding the standard supplement for a maximum period of 6 weeks (2 weeks pre-lambing and 4 weeks into lambing, by which time the majority of ewes would have lambed) would cost less than \$0.50/hd. Even a slight (1%) improvement in lamb survival would be sufficient to justify this expense at current wool and meat prices. Supplementation could also be expected to reduce ewe mortality in flocks where a significant number of ewes were clinically deficient in Ca (reducing milk fever incidence) and Mg (reducing grass tetany incidence), or sub-clinically deficient in these minerals (by reducing the risk of twin-lamb disease and other metabolic disorders). Furthermore, if the weight advantage (>2kg 4 weeks of age) of twin lambs born to ewes consuming higher levels of Ca and Mg in the intensive pen study is replicated in flocks supplemented with these minerals, this weight advantage alone could be expected to translate to improved weaning weights, improving weaner survival and time to reach sale and/or joining weights, which could justify the investment alone independent of any effect on lamb or ewe survival.

10. IMPACT OF WOOL INDUSTRY – NOW & IN 5 YEARS' TIME

Although this project was unable to demonstrate any improvement in lamb survival by supplementation of Mg and Ca to grazing pregnant ewes, the project did demonstrate that many late pregnant ewes are likely to be deficient in Ca and Mg in flock grazing common southern Australian pastures. There is good evidence, based on the comprehensive review of the literature, and improvement in mechanisms likely to impact on lamb survival when the Ca and Mg status of ewes is improved under controlled conditions, that supplementation is likely to be effective in improving lamb survival and early weight gain (and possibly reducing ewe mortality), especially in twin-bearing flocks.

High meat and wool prices have increased producer interest in improved nutritional management of flocks. Recommending the supplementation of Ca and Mg as part of best management recommendations, especially for twin-bearing ewes grazing grass-dominant (including cereal crop) pastures as a low cost insurance measure, should see an improvement in lamb survival and also early lamb growth rates. It is hard to estimate the size of

the survival impact, as the response will vary widely between individual flocks, and our data does not enable us to estimate the likely size of the impact. However, at a national scale the impact is likely to be modest (less than 5%), even though in individual flocks it could be profound. Given the observed effects of higher Ca and Mg levels on improved ewe energy status and lamb immunity, the practice should be promoted on welfare grounds, especially given the low cost of supplementation. Further work is recommended to understand factors affecting supplement intake so that guidelines can be developed for ensuring adequate supplement intake.

The size of the increase in twin-lamb growth rate to 4 weeks of age achieved through supplementation, based on our results, could be as high as 20%. Adopted at scale, if the early growth of twins translates to higher weaning weights, this will result in improvements in weaner survival. Furthermore, for lambs destined for sale, this will translate to higher sale weights and/or earlier sale dates, leading to improvements in profit. Further work would be required to quantify the potential size of this impact, but given the low cost of supplementation and the increase in early growth, a favourable benefit: cost ratio is expected

11. CONCLUSIONS AND RECOMMENDATIONS

These studies indicate that sub-clinical hypocalcaemia and hypomagnesaemia commonly occur at significant levels in late-pregnant ewes grazing common pastures. Whilst mineral supplementation did not improve lamb survival in any of the grazing studies, the pen study, where intake of supplement could be controlled, did show that mineral supplementation can improve the metabolic status of ewes. An improved ability to regulate energy balance may reduce the risk of inducing metabolic disease, especially when ewes are challenged nutritionally or otherwise. In addition, Ca and Mg supplementation did result in much lower contraction and parturition duration (although not statistically significant) for second lambs from twin bearing ewes, which could be expected to have positive effects on reducing dystocia.

Furthermore, mineral supplementation of ewes during late pregnancy and lactation resulted in an improved immune response in lambs, which has the potential to lead to some improvement in lamb survival. The greater live weight (at 4 weeks of age) of lambs from supplemented groups suggests that supplementation on this basis alone may be worthwhile. However, it is unknown whether the almost 2 kg greater weight of lambs at 4 weeks of age from supplemented groups (Ca, Mg and Ca+Mg) in the pen feeding experiment (where mineral intake was controlled) could be achieved in ewes supplemented with minerals at pasture, and if so whether this weight advantage could be maintained over time. If lambs from supplemented ewes were heavier at weaning, this would improve weaner survival (or reduce costs to reach weaning weights), and for lambs destined for processing, result in shorter time to reach target weights, resulting in cost savings. Further work is recommended to determine if supplementation of Ca and Mg at pasture can improve lamb weight gains.

Remaining challenges include understanding factors affecting supplement intake of mineral supplements – intake was variable across the sites investigated in this project, which may be related to feed on offer. Both forms of supplement used in this project (standard and low DCAD) appeared palatable, although feed manufacturers will often include other additives (e.g. dextrose) to improve intake, although this also increases the cost. If supplement intake can be cost-effectively increased, further work could be undertaken to determine whether higher levels of Ca and Mg supplementation than used in the present suite of experiments could lead to improvements in survival.

Given the low cost of the calcium and magnesium supplements used in this project (<\$0.02/hd/day), supplementation of grazing ewes during lambing can be recommended as a risk management strategy to minimise the risk of metabolic disorders, improve lamb immunity and early weight gains. While we were unable to demonstrate any significant effect on lamb survival from supplementation, improvement in these traits could be expected to improve lamb survival in some flocks.

12. BIBLIOGRAPHY

- Abuela, A, Hernández, J, Benedito, JL, Castillo, C (2015) The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. *Journal of Animal Physiology and Animal Nutrition* **99**, 1003-1016.
- Achmadi, J, Sano, H, Terashima, Y (2001) Effect of hypomagnesemia and cold exposure on tissue responsiveness to insulin in sheep given a low magnesium and high potassium diet. *Domestic Animal Endocrinology* **20**, 101-108.
- Albera, E, Kankofer, M (2011) The comparison of antioxidative/oxidative profile in blood, colostrum and milk of early post-partum cows and their newborns. *Reproduction in Domestic Animals*, **46**, 763-769. Retrieved from <http://dx.doi.org/10.1111/j.1439-0531.2010.01737.x>
- Ali, AMH (2011) Causes and management of dystocia in small ruminants in Saudi Arabia. *Journal of Agricultural and Veterinary Sciences* **4**, 95-108.
- Allard, B, Long, E, Block, E, Zhao, X (1999) Dependence of superoxide anion production on extracellular and intracellular calcium ions and protein kinase C in PMA-stimulated bovine neutrophils. *Canadian Journal of Veterinary Research*, **63**, 13-17. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1189509/>
- Allen, WM, Sansom, BF (1986) Metabolic Disorders In 'Current Veterinary Therapy: Food Animal Practice ' (Ed. Howard JL.) Vol. 2 pp. 311-322. (W.B. Saunders Philadelphia)
- Allworth, M, Wrigley, H, Cowling, A (2016) Fetal and lamb losses from pregnancy scanning to lamb marking in commercial sheep flocks in southern New South Wales. *Animal Production Science Online*.
- Andrews, A (1997) Pregnancy toxemia in the ewe. *In Practice* **19**, 306-314. Available at <http://inpractice.bmj.com/content/19/6/306.abstract> [Accessed November 16, 2010].
- Annisson, EF, Lindsay, DB, Nolan, JV (2002) Digestion and metabolism. In 'Sheep Nutrition.' (Eds M Freer, H Dove.) pp. 95-118. (CAB International: Wallingford, UK)
- Arnold, GW, Morgan, PD (1975) Behaviour of the ewe and lamb at lambing and its relationship to lamb mortality. *Applied Animal Ethology* **2**, 25-46.
- Arthur, GH (1975) 'Veterinary Reproduction and Obstetrics.' (Baillière Tindall: London)
- Baird, N, Pugh, DG (2012a) 'Sheep & Goat Medicine.' (Elsevier Health Sciences: Saint Louis, United States)
- Baird, N, Pugh, DG (2012b) 'Sheep & Goat Medicine.' (Elsevier Health Sciences: Saint Louis)
- Beardsworth, L, Beardsworth, P, Care, A (1989) The effect of ruminal phosphate concentration on the absorption of calcium, phosphorus and magnesium from the reticulo-rumen of the sheep. *British Journal of Nutrition* **61**, 715-723.

- Beck, N, Webster, SK (1976) Effects of acute metabolic acidosis on parathyroid hormone action and calcium mobilization. *American Journal of Physiology* **230**, 127-131.
- Beede , DK (1992) The DCAD concept: Transition rations for dry pregnant cows. *Feedstuffs* **64**, 12.
- Berger, LL (1992) Grass Tetany: Causes and Prevention. *Salt & Trace Minerals* **24**, Available at <http://www.saltinstitute.org/content/download/357/1941/file/stm-sept-1992.pdf>
- Bhanugopan, MS, Fulkerson, WJ, Hyde, M, Fraser, DR (2015) Effect of dietary potassium supplementation on the calcium absorption capacity in the rumen and abomasum and fractional excretion of urinary minerals in sheep. *Animal Production Science* **55**, 508-514.
- Bird, P, Lynch, J, Obst, J (1984) Effect of shelter on plant and animal production. *Animal Production in Australia* **15**, 270-273.
- Blaine, J, Chonchol, M, Levi, M (2014) Renal control of calcium, phosphate, and magnesium homeostasis. *Clinical Journal of the American Society of Nephrology, Online*, doi: 10.2215/CJN.09750913CJASN
- Bloom, S, Edwards, A (1975) The release of pancreatic glucagon and inhibition of insulin in response to stimulation of the sympathetic innervation. *The Journal of Physiology* **253**, 157.
- Blumer, CC, Madden, FJ, Walker, DJ (1939) Hypocalcaemia, grass tetany or grass staggers in sheep. *Australian Veterinary Journal* **15**, 24-27.
- Boass, A, Garner, SC, Schultz, VL, Toverud, SU (1997) Regulation of serum calitriol by serum ionized calcium in rats during pregnancy and lactation. *Journal of Bone Mineral Research* **12**, 909-914.
- Bohman, VR, Horn, FP, Stewart, BA, Mathers, AC, Grunes, DL (1983) Wheat pasture poisoning. I. An evaluation of cereal pastures as related to tetany in beef cows. *Journal of Animal Science* **57**, 1352-1363.
- Bozos, C, Mavrogianni, V, Fthenkis, G (2011) Treatment and control of Peri-Parturient metabolic diseases: Pregnancy toxemia, Hypocalcemia, Hypomagnesemia. *Veterinary Clinics of North America : Food Animal Practice* **27**, 105 -113.
- Braithwaite, GD (1972) The effect of ammonium chloride on calcium metabolism in sheep. *British Journal of Nutrition* **27**, 201-209.
- Braithwaite, GD (1976) Reviews of the progress of dairy science: calcium and phosphorus metabolism in ruminants with special reference to parturient paresis. *Journal of Dairy Research* **43**, 501-520.
- Braithwaite, GD (1978) The effect of dietary calcium intake of ewes in pregnancy on their Ca and phosphorus metabolism in lactation. *British Journal of Nutrition* **39**, 213-218.
- Braithwaite, GD (1980) The effect of dose rate of 1- α -hydroxycholecalciferol on calcium and phosphorus metabolism in sheep. *British Journal of Nutrition* **44**, 183-191.
- Braithwaite, GD (1983) Calcium and phosphorus requirements of the ewe during pregnancy and lactation. 1. Calcium. *British Journal of Nutrition* **50**, 711-722.
- Braithwaite, GD, Glascock, RF, Riazuddin, S (1969) Calcium metabolism in lactating ewes. *British Journal of Nutrition* **23**, 827-834.

- Braithwaite, GD, Glascock, RF, Riazuddin, S (1970) Calcium metabolism in pregnant ewes. *British Journal of Nutrition* **24**, 661-670.
- Breazile, JE (1994) Skeletal muscle physiology. In 'Duke's physiology of domestic animals.' (Eds MJ Swenson, WC Reece.) pp. 855-873. (Comstock Publishing Association: Ithaka, NY)
- Bronner, F (1987) Intestinal Calcium-Absorption - Mechanisms and Applications. *Journal of Nutrition* **117**, 1347-1352.
- Brookes, IM, Nicol, AM (2007) The protein requirements of grazing livestock. In 'Pasture and Supplements for Grazing Animals.' (Eds PV Rattray, IM Brookes, AM Nicol.) Vol. Occasional Publication No. 14 pp. 16. (New Zealand Society of Animal Production: Hamilton, New Zealand)
- Cabrera Estrada, JI, Delagarde, R, Faverdin, P, Peyraud, JL (2004) Dry matter intake and eating rate of grass by dairy cows is restricted by internal, but not external water. *Animal Feed Science and Technology* **114**, 59-74.
- Caple, IV, Azuolas, JK, Nugent, GF (1985) Assessment of iodine status and thyroid function of sheep and goats kept under pastoral conditions. In 'Trace Element Metabolism in Man and Animals. Proceedings of the Fifth International Symposium on Trace Element Metabolism in Man and Animals (TEMA-5).' (Eds CF Mills, I Bremner, JK Chesters.) pp. 609-613. (Commonwealth Agricultural Bureaux: Slough)
- Caple, IW, Babacan, E, Pham, TT, Heath, JA, Grant, IM, Vizard, AL, Cameron, SJ, Allworth, MB (1988a) Seasonal vitamin D deficiency in sheep in south-eastern Australia. *Proceedings of the Australian Society of Animal Production* **17**, 379.
- Caple, IW, Heath, JA, Grant, IM (1988b) Calcium requirements of sheep during pregnancy, lactation and growth. *Proceedings of the Nutrition Society of Australia* **13**, 33-40.
- Care, A, Brown, R, Farrar, A, Pickard, D (1984) Magnesium absorption from the digestive tract of sheep. *Quarterly Journal of Experimental Physiology* **69**, 577-587.
- Chapinal, N, Carson, M, Duffield, TF, Capel, M, Godden, S, Overton, M, Santos, JEP, LeBlanc, SJ (2011) The association of serum metabolites with clinical disease during the transition period. *Journal of Dairy Science* **94**, 4897-4903.
- Chen, TC, Castillo, L, Koryckad, M, Deluca, HF (1974) Role of Vitamin-D Metabolites in Phosphate Transport of Rat Intestine. *Journal of Nutrition* **104**, 1056-1060.
- Christley, R, Morgan, K, Parkin, T, French, N (2003) Factors related to the risk of neonatal mortality, birth-weight and serum immunoglobulin concentration in lambs in the UK. *Preventive Veterinary Medicine* **57**, 209-226.
- Cinar, V, Polat, Y, Mogulkoc, R, Nizamlioglu, M, Baltaci, A K (2008) The effect of magnesium supplementation on glucose and insulin levels of tae-kwan-do sportsmen and sedentary subjects. *Pakistan Journal of Pharmaceutical Sciences*, **21**, 237-240.

- Clark, DA, Woodward, SL (2007) Supplementation of dairy cows, beef cattle and sheep grazing pasture. In 'Pasture and Supplements for Grazing Animals.' (Eds PV Rattray, IM Brookes, AM Nicol.) Vol. Occasional Publication No. 14 pp. 15. (New Zealand Society of Animal Production: Hamilton, New Zealand)
- Cloete, SWP, Scholtz, AJ, Ten Hoope, JM, Lombard, PJA, Franken, MC (1998) Ease of birth relation to pelvic dimensions, litter weight and conformation of sheep. *Small Ruminant Research* **31**, 51-60.
- Cockcroft, P, Whiteley, P (1999) Hypocalcaemia in 23 ataxic/recumbent ewes: clinical signs and likelihood ratios. *Veterinary Record* **144**, 529-32.
- Coffman, J (1980) Calcium and phosphorus physiology and pathophysiology. *Equine Practice* **9**, 93-96.
- Court, J, Hides, S, Webb-Ware, J (2010) 'Sheep Farming for Meat and Wool.' (CSIRO Publishing: Collingwood)
- CSIRO (2007) 'Nutrient Requirements of Domesticated Ruminants.' (CSIRO Publishing: Melbourne, Australia)
- Cunningham, J, Klein, B (Ed. B Klein (2013) 'Cunningham's Textbook of Veterinary Physiology.' (Elsevier/Saunders: St. Louis, Mo)
- Curry, DL, Joy, RM, Holley, DC, Bennett, LL (1977) Magnesium modulation of glucose-induced insulin secretion by the perfused rat pancreas*. *Endocrinology* **101**, 203-208.
- Curtis, C, Erb, H, Sniffen, C, Smith, R, Powers, P, Smith, M, White, M, Hillman, R, Pearson, E (1983a) Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. *Journal of the American Veterinary Medical Association* **183**, 559-561.
- Curtis, CR, Erb, HN, Sniffen, CJ, Smith, RD (1983b) Epidemiology of parturient paresis: dry cow management and feeding in Holstein cows. *Journal of Dairy Science* **66**, 270.
- Curtis, KMS (2014) Stocktake of the Australian sheep flock. Meat & Livestock Australia Ltd No. BLSM.0055.
- Dalton, DC, Knight, TW, Johnson, DL (1980) Lamb survival in sheep breeds on New Zealand hill country. *New Zealand Journal of Agricultural Research* **23**, 167-173.
- Daniel, RCW (1983) Motility of the rumen and abomasum during hypocalcaemia. *Canadian Journal of Comparative Medicine* **47**, 276-280.
- de Baaij, JHF, Hoenderop, JGJ, Bindels, RJM (2012) Regulation of magnesium balance: lessons learned from human genetic disease. *Clinical Kidney Journal*, **5**, 15-24. doi:10.1093/ndtplus/sfr164
- de Haan, HH, Gunn, AJ, Williams, CE, Heymann, MA, Gluckman, PD (1997) Magnesium sulfate therapy during asphyxia in near-term fetal lambs does not compromise the fetus but does not reduce cerebral injury. *American Journal of Obstetrics and Gynecology* **176**, 18-27.
- Dennis, SM (1974a) Perinatal lamb mortality in Western Australia. 1. General procedures and results. *Australian Veterinary Journal* **50**, 443-449.
- Dennis, SM (1974b) Perinatal lamb mortality in Western Australia. 2. Non-infectious conditions. *Australian Veterinary Journal* **50**, 450-453.
- Dove, H (2007) Mineral nutrition of sheep grazing dual-purpose wheats. In 'Grains Research Technical Update, Wagga Wagga, NSW.' (Ed. D Kaminskas, Rawlings, S.) pp. 71-75.

- Dove, H, Kelman, W (2015) Liveweight gains of young sheep grazing dual-purpose wheat with sodium and magnesium supplied as direct supplement, or with magnesium supplied as fertiliser. *Animal Production Science* **55**, 1217-1229.
- Dove, H, Kelman, WM, Kirkegaard, JA, Sprague, SJ (2012) Impact of magnesium–sodium supplementation on liveweight gains of young sheep grazing dual-purpose cereal or canola crops. *Animal Production Science* **52**, 1027-1035.
- Dove, H, Masters, DG, Thompson, AN (2016) New perspectives on the mineral nutrition of livestock grazing cereal and canola crops. *Animal Production Science* **56**, 1350-1360.
- Dove, H, McMullen, KG (2009) Diet selection, herbage intake and liveweight gain in young sheep grazing dual-purpose wheats and sheep responses to mineral supplements. *Animal Production Science* **49**, 749-758.
- DPI, N (2008) Metabolic Diseases of Sheep. *Animal Health Surveillance* Available at http://www.dpi.nsw.gov.au/_data/assets/pdf_file/0015/252033/AHN-Sept-2008.pdf [Accessed 20.1.11].
- Dutra, F, Banchero, G (2011) Polwarth and Texel ewe parturition duration and its association with lamb birth asphyxia. *Journal of Animal Science* **89**, 3069-3078.
- Dutra, F, Quintans, G, Banchero, G (2007) Lesions in the central nervous system associated with perinatal lamb mortality. *Australian Veterinary Journal* **85**, 405-413.
- Dwyer, CM (2003) Behavioural development in the neonatal lamb: effect of maternal and birth-related factors. *Theriogenology* **59**, 1027-1050.
- Dwyer, CM (2014) Maternal behaviour and lamb survival: from neuroendocrinology to practical application. *Animal* **8**, 102-112.
- Dwyer, CM, Bünger, L (2012) Factors affecting dystocia and offspring vigour in different sheep genotypes. *Preventive Veterinary Medicine* **103**, 257-264.
- Dwyer, CM, Conington, J, Corbiere, F, Holmøy, IH, Muri, K, Nowak, R, Rooke, J, Vipond, J, Gautier, J-M (2015) Invited review: Improving neonatal survival in small ruminants: science into practice. *Animal* **10**, 449-59.
- Dwyer, CM, Lawrence, AB, Brown, HE, Simm, G (1996) Effect of ewe and lamb genotype on gestation length, lambing ease and neonatal behaviour of lambs. *Reproduction, Fertility and Development* **8**, 1123-1129.
- Elias, E, Shainkin-Kestenbaum, R (1990) Hypocalcaemia and serum levels of inorganic phosphorus, magnesium parathyroid and calcitonin hormones in the last month of pregnancy in Awassi fat-tail ewes. *Reproduction, Nutrition and Development* **30**, 693-699.
- Elliott, DA, Rizack, MA (1974) Epinephrine and adrenocorticotrophic hormone-stimulated magnesium accumulation in adipocytes and their plasma membranes. *Journal of Biological Chemistry* **249**, 3985-3990.
- Elliott, M, 2009. Grass tetany in cattle. Primefact 420. New South Wales Department of Primary Industry.

- English PB, Hogan AE (1979) A comparison of urine specific gravity and osmolality in sheep. *Australian Veterinary Journal* **55**, 584-586.
- Epstein, G, Fanksa, R, Grodsky, GM (1978) The effect of potassium and valinomycin on insulin and glucagon secretion in the perfused rat pancreas. *Endocrinology* **103**, 2207-2215.
- Espino, L, Guerrero, F, Suarez, ML, Santamarina, G, Goicoa, A, Fidalgo, LE (2003) Long-term effects of dietary anion–cation balance on acid–base status and bone morphology in reproducing ewes. *Journal of Veterinary Medicine Series A* **50**, 488-495.
- Everett-Hincks, JM, Dodds, KG (2008) Management of maternal-offspring behavior to improve lamb survival in easy care sheep systems *Journal of Animal Science* **86**, E259-E270.
- Fawcett, W, Haxby, E, Male, D (1999) Magnesium: physiology and pharmacology. *British Journal of Anaesthesia* **83**, 302-320.
- Field, A, Suttle, N, Nisbet, D (1975) Effect of diets low in calcium and phosphorus on the development of growing lambs. *The Journal of Agricultural Science* **85**, 435-442.
- Fogarty, N, Mulholland, J (2014) Seasonal reproductive performance of crossbred ewes in intensive lamb-production systems. *Animal Production Science* **54**, 791-801.
- Fogarty, NM, Hall, DG, Holst, PJ (1992) The effect of nutrition in mid pregnancy and ewe liveweight change on birth weight and management for lamb survival in highly fecund ewes. *Australian Journal of Experimental Agriculture* **32**, 1-10.
- Fonsêca, VFC, Saraiva, EP, Pimenta Filho, EC, Furtado, DA, Mariz, TMA, Silva, AL, Almeida, MEV, Pinheiro, AC (2014) Influence of the climatic environment and duration of labor on the mother-offspring interaction in Morada Nova sheep. *Journal of Animal Science* **92**, 4123-4129.
- Foster, A, Livesey, C, Edwards, GR (2007) Magnesium disorders in ruminants. *In Practice* **29**, 534-539. Available at <http://inpractice.bvapublications.com/cgi/reprint/29/9/534>
- Fowler, D (2007) Lamb marking performance for ultrasound scanned ewes in Australian sheep flocks. MLA Final Report. Project Code AHW.131. Meat & Livestock Australia Ltd, North Sydney, NSW. Available at <http://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Animal-Health-and-Biosecurity/Lamb-marking-performance-for-ultrasound-scanned-ewes-in-Australian-sheep-flocks/131>.
- Fraser, DR (1980) Regulation of the metabolism of vitamin D. *Physiological Reviews* **60**, 551-613.
- Fraser, DR (1995) Vitamin D. *Lancet* **345**, 104-107.
- Fredeen, A, DePeters, E, Baldwin, R (1988) Characterization of acid-base disturbances and effects on calcium and phosphorus balances of dietary fixed ions in pregnant or lactating does. *Journal of Animal Science* **66**, 159-173.
- Fredeen, AH (1990) Effects of calcium loss and high dietary calcium and potassium on calcium kinetics and magnesium balance in sheep fed low magnesium diets. *Canadian Journal of Animal Science* **70**, 1109-1117.
- Freer, M, Dove, H, Nolan, JV (Eds) (2007) 'Nutrient Requirements of Domesticated Livestock.' (CSIRO Publishing: Melbourne).

- Gaál, T, Ribiczeyné-Szabó, P, Stadler, K, Jakus, J, Reiczigel, J, Kövér, P, . . . Sümeghy, L (2006) Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **143**, 391-396. Retrieved from <http://www.sciencedirect.com/science/article/pii/S109649590500312X>
- Geenty, K, Brien, F, Hinch, G, Dobos, R, Refshauge, G, McCaskill, M, Ball, A, Behrendt, R, Gore, K, Savage, D (2014) Reproductive performance in the Sheep CRC Information Nucleus using artificial insemination across different sheep-production environments in southern Australia. *Animal Production Science* **54**, 715-726.
- George, JM (1976) The incidence of dystocia in Dorset Horn ewes. *Australian Veterinary Journal* **52**, 519-523.
- Goff, JP (1989) Calcium homeostasis challenged during lactation. *Feedstuffs* **61**, 14-16.
- Goff, JP (2000) Pathophysiology of calcium and phosphorus disorders. *Veterinary Clinics of North America, Food Animal Practice* **16**, 319-337.
- Goff, JP (2006) Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. *Animal Feed Science and Technology* **126**, 237-257.
- Goff, JP, Horst, RL (1997a) Effects of the addition of potassium or sodium, but not calcium, to prepartum rations on milk fever in dairy cows. *Journal of Dairy Science* **80**, 176-186.
- Goff, JP, Horst, RL (1997b) Physiological changes at parturition and their relationship to metabolic disorders^{1,2}. *Journal of Dairy Science* **80**, 1260-1268.
- Goff, JP, Horst, RL (1998) Use of hydrochloric acid as a source of anions for prevention of milk fever. *Journal of Dairy Science* **81**, 2874-2880.
- Goff, JP, Reinhardt, TA, Horst, RL (1991) Enzymes and factors controlling vitamin D metabolism and action in normal and milk fever cows. *Journal of Dairy Science* **74**, 4022-4032.
- Gordon, AM (1989) Molecular basis of contraction. In 'Textbook of Physiology: Excitable Cells and Neurophysiology.' (Eds HD Patton, AF Fuchs, B Hille.) pp. 177-178. (W.B. Saunders Co.: Philadelphia)
- Goubern, M, Rayssiguier, Y, Miroux, B, Chapey, M, Ricquier, D, Durlach, J (1993) Effect of acute magnesium deficiency on the masking and unmasking of the proton channel of the uncoupling protein in rat brown fat. *Magnesium Research* **6**, 135-143.
- Grace, N, Ulyatt, M, Macrae, J (1974) Quantitative digestion of fresh herbage by sheep III. The movement of Mg, Ca, P, K and Na in the digestive tract. . *Journal of Agricultural science* **82**, 321-330.
- Grant, IM, Bingham, AM, Caple, IW (1992) Acid-base balance and susceptibility of ewes to hypocalcaemia. *Proceedings of the Australian Society of Animal Production* **19**, 412-415.

- Grant, IM, Foot, JZ, Brockus, MA, Bingham, AM, Caple, IW (1988) Factors affecting plasma calcium and phosphorus concentrations in grazing ewes in spring and autumn lambing flocks in Victoria. *Proceedings of the Australian Society of Animal Production* **17**, 194-197.
- Greene, L, Webb, K, Fontenot, J (1983a) Effect of potassium level on site of absorption of magnesium and other macroelements in sheep. *Journal of Animal Science* **56**, 1214-1221.
- Greene, LW, Fontenot, JP, Webb, KE (1983b) Site of magnesium and other macromineral absorption in steers fed high levels of potassium. *Journal of Animal Science* **57**, 503-510.
- Greene, LW, Fontenot, JP, Webb, KE, Jr. (1983c) Effect of dietary potassium on absorption of magnesium and other macroelements in sheep fed different levels of magnesium. *Journal of Animal Science* **56**, 1208-1213.
- Grings, EE, Male, JR (1987) Effects of potassium on macromineral absorption in sheep fed wheat straw-based diets. *Journal of Animal Science* **64**, 872-879.
- Grohn, YT, Fubini, SL, Smith, DF (1990) Use of a multiple logistic regression model to determine prognosis of dairy cows with right displacement of the abomasum or abomasal volvulus. *American Journal of Veterinary Research* **51**, 1895-1899.
- Hansard, S, Comar, C, Plumlee, M (1954) The effects of age upon calcium utilisation and maintenance requirements in the bovine. *Journal of Animal Science* **13**, 25-35.
- Harmeyer, J, Schlumbohm, C (2006) Pregnancy impairs ketone body disposal in late gestating ewes: Implications for onset of pregnancy toxemia. *Research in Veterinary Science* **81**, 254-64.
- Harris, DJ, Lambell, RG, Oliver, CJ (1983) Factors predisposing dairy and beef cows to grass tetany. *Australian Veterinary Journal* **60**, 230-234.
- Hartley, WJ, Kater, JC (1964) Perinatal disease conditions of sheep in New Zealand. *New Zealand Veterinary Journal* **12**, 49-57.
- Haughey, KG (1980) The role of birth in the pathogenesis of meningeal haemorrhage and congestion in newborn lambs. *Australian Veterinary Journal* **56**, 49-56.
- Haydock, KP, NH Shaw (1975). The comparative yield method for estimating dry matter yield of pasture. *Australian Journal of Experimental Agriculture and Animal Husbandry* **15**, 663-670.
- Henze, P, Bickhardt, K, Fuhrmann, H, Sallmann, H (1998) Spontaneous pregnancy toxemia (ketosis) in sheep and the role of insulin. *Journal of Veterinary Medicine Series A* **45**, 255-266.
- Herd, RP (1965) The relationship between hypocalcaemia and hypomagnesaemia in sheep. *Australian Veterinary Journal* **41**, 385-386.
- Herd, RP (1966a) Fasting in relation to hypocalcaemia and hypomagnesaemia in lactating cows and ewes. *Australian Veterinary Journal* **42**, 269-272.
- Herd, RP (1966b) Grass tetany in sheep. *Australian Veterinary Journal* **42**, 160-164.
- Hergenhan, RL, Hinch, GN, Ferguson, DM (2014) Sire effects on neonatal lamb vigour and following-behaviour. *Animal Production Science* **54**, 745-752.

- Hibbs, JW, Conrad, HR (1966) Re-Evaluation of Nutrient Allowances for High-Producing Cows .2. Calcium Phosphorus and Vitamin D. *Journal of Dairy Science* **49**, 243.
- Hight, GK, Jury, KE (1970) Hill country sheep production. *New Zealand Journal of Agricultural Research* **13**, 735-752.
- Hinch, G, Brien, F (2014) Lamb survival in Australian flocks: a review. *Animal Production Science* **54**, 656-666.
- Hinch, G, Lynch, J, Nolan, J, Leng, R, Bindon, B, Piper, L (1996) Supplementation of high fecundity Border Leicester x Merino ewes with a high protein feed: its effect on lamb survival. *Australian Journal of Experimental Agriculture* **36**, 129-136.
- Hindson, JC (1961) Ringwomb in the ewe. *Veterinary Record* **73**, 85-86.
- Hocking Edwards JE, Masters, DG, Winslow, E, Hancock, S, Thompson, AN, Refshauge, G, McGrath, SR, Robertson, SM, Bhanugopan, MS, Friend MA (2018) Calcium and magnesium status of pregnant ewes grazing southern Australian pastures. *Animal Production Science*, **58**, 1515-1521.
- Hogan, JS, Weiss, WP, Smith, KL (1993) Role of vitamin E and selenium in host defense against mastitis. *Journal of Dairy Science* **76**, 2796-2803.
- Höller, H, Breves, G, Gerdes, H, Kocabatmaz, M (1988) Flux of calcium across the sheep rumen wall *in vivo* and *in vitro*. *Quarterly Journal of Experimental Physiology* **73**, 609-618.
- Holst, P, Fogarty, N, Stanley, D (2002) Birth weights, meningeal lesions, and survival of diverse genotypes of lambs from Merino and crossbred ewes. *Crop and Pasture Science* **53**, 175-181.
- Holst, PJ, 2004. Lamb autopsy - notes on a procedure for determining cause of death. NSW Agriculture, 4-24.
- Hou, WX, Cheng, SY, Liu, ST, Shi, BM, Shan, AS (2014) Dietary supplementation of magnesium sulfate during late gestation and lactation affects the milk composition and immunoglobulin levels in sows. *Asian Australasian Journal of Animal Science* **27**, 1469-1477.
- Hove, K (1978) Insulin secretion in lactating cows: responses to glucose infused intravenously in normal, ketonemic, and starved animals. *Journal of Dairy Science* **61**, 1407-1413.
- Hove, K (1984) Intestinal radiocalcium absorption in the goat: measurement by a double-isotope technique. *British Journal of Nutrition* **51**, 145-156.
- Huber, TL, Wilson, RC, Stattelman, AJ, Goetsch, DD (1981) Effect of hypocalcaemia on motility of the ruminant stomach. *American Journal of Veterinary Research* **42**, 1488-1490.
- Hughes, KL, Hartley, WJ, Haughey, KG, McFarlane, D (1964) A study of perinatal mortality of lambs from the Oberon, Orange and Monaro districts of N.S.W. *Proceedings of the Australian Society of Animal Production* **5**, 92-99.

- Iggo, A (1983) Activity of peripheral nerves and junctional regions. In 'Duke's Physiology of Domestic Animals.' (Ed. MJ Swenson.) pp. 613-614. (Comstock Publishing Associates: Ithaca, NY)
- Ingraham, R, Kappel, L, Morgan, E, Srikandakumar, A (1987) Correction of subnormal fertility with copper and magnesium supplementation. *Journal of Dairy Science* **70**, 167-180.
- Ishizuka, J, Bold, RJ, Townsend Jr, C, Thompson, J (1994) *In vitro* relationship between magnesium and insulin secretion. *Magnesium research: official organ of the International Society for the Development of Research on Magnesium* **7**, 17-22.
- Jableck, CK (1993) Disorders of neuromuscular transmission and muscles. In 'Clinical Electromyography.' (Eds WF Brown, CF Bolton.) pp. 677-678. (Butterworth-Heinemann: Boston)
- Jacobs, JL, Rigby, SE (1999) Minerals in Dairy Pastures in Victoria. Department of Natural Resources and Environment, Warrnambool, Australia.
- Jaconi MEE , Lew, D P, Carpentier, JL, Magnusson, KE, Sjogren, M, Stendahl, O (1990) Cytosolic free calcium elevation mediates the phagosome-lysosome fusion during phagocytosis in human neutrophils. *The Journal of Cell Biology*, **110**, 1555-1564. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2200167/>
- Jawor, P, Huzzey, J, LeBlanc, S, von Keyserlingk, M (2012) Associations of sub-clinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing behaviors around parturition in Holstein cows. *Journal of Dairy Science* **95**, 1240-1248.
- Jefferies BC (1961) Body condition scoring and its use in management. *Tasmanian Journal of Agriculture* **32**, 19-21.
- Jefferson, PG, Mayland, HF, Asay, KH, Berdahl, JD (2001) Variation in mineral concentration and grass tetany potential among Russian Wildrye accessions. *Crop Science* **41**, 543-548.
- Jonsson, NN, Daniel, RCW (1997) Effects of hypocalcaemia on blood flow to the ovaries of the sheep. *Journal of Veterinary Medicine Series A* **44**, 281-287.
- Joyce, JP, Brunswick, LCF (1975) Sodium supplementation of sheep and cattle fed lucerne. *New Zealand Journal of Experimental Agriculture* **3**, 299-304.
- Jubb, KVF, Huxtable, CR (1993) The nervous system. In 'Pathology of Domestic Animals, 4th Edition.' (Eds KVF Jubb, PC Kennedy, N Palmer.) Vol. 1 pp. 323-336. (Academic Press: San Diego, CA)
- Judson, GJ, McFarlane, JD (1998) Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. *Australian Journal of Experimental Agriculture* **38**, 707-723.
- Kahn, CM, Line, S, Co., M, 2010. The Merck Veterinary Manual. Whitehouse Station, N.J: Merck & Co,
- Kelly, RW (1992) Lamb mortality and growth to weaning in commercial Merino flocks in Western Australia. *Australian Journal of Agricultural Research* **43**, 1399-1416.
- Kempt, A, 't Hart, J (1957) Grass tetany in grazing milking cows. *Netherlands Journal of Agricultural Science* **5**, 4-17.

- Kenyon, P, Morris, S, Revell, D, McCutcheon, S (2003) Shearing during pregnancy—review of a policy to increase birthweight and survival of lambs in New Zealand pastoral farming systems. *New Zealand Veterinary Journal* **51**, 200-207.
- Khorasani, GR, Armstrong, DG (1990) Effect of sodium and potassium level on the absorption of magnesium and other macrominerals in sheep. *Livestock Production Science* **24**, 223-235.
- Kimura, J (1989) 'Neuromuscular Diseases Characterized by Abnormal Muscle Activity: Electrodiagnosis in Diseases of Nerve and Muscle, Principles and Practice.' (F.A. Davis Co.: Philadelphia)
- Kimura, K, Reinhardt, T, Goff, J (2006) Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. *Journal of Dairy Science* **89**, 2588-2595.
- Kleemann, DO, Walker, SK (2005) Fertility in South Australian commercial Merino flocks: sources of reproductive wastage. *Theriogenology* **63**, 2075-2088.
- Kozakai, T, Uozumi, N, Katoh, K, Obara, Y (2002) Dietary magnesium increases calcium absorption of ovine small intestine *in vivo* and *in vitro*. *Reproduction, Nutrition and Development* **42**, 25-33.
- Kremer, R, Barbato, G, Rista, L, Rosés, L, Perdígón, F (2010) Reproduction rate, milk and wool production of Corriedale and East Friesian x Corriedale F1 ewes grazing on natural pastures. *Small Ruminant Research* **90**, 27-33.
- Laires, M, Monteiro, C (2008) Exercise, magnesium and immune function. *Magnesium Research* **21**, 92-96.
- Lane, J, Jubb, T, Shephard, R, Webb-Ware, J, Fordyce, G (2015) Priority list of endemic diseases for the red meat industries. Meat and Livestock Australia, North Sydney.
- Larsen, JWA, Constable, PD, Napthine, DV (1986) Hypocalcaemia in ewes after a drought. *Australian Veterinary Journal* **63**, 25-26.
- Las, JE, Odongo, NE, Lindinger, MI, Aizahal, O, Shoveller, AK, Matthews, JC, McBride, BW (2007) Effects of dietary strong acid anion challenge on regulation of acid-base balance in sheep. *Journal of Animal Science* **85**, 2222-2229.
- Leclercq-Meyer, V, Marchand, J, Malaisse, W (1973) The effect of calcium and magnesium on glucagon secretion. *Endocrinology* **93**, 1360-1370.
- Lentz, D, Madsen, F, Miller, J, Hansard, S (1976) Effect of potassium and hypomagnesemia on insulin in the bovine. *Journal of Animal Science* **43**, 1082-1087.
- Liesegang, A (2008) Influence of anionic salts on bone metabolism in periparturient dairy goats and sheep. *Journal of Dairy Science* **91**, 2449-2460.
- Liesegang, A, Riner, K, Boos, A (2007) Effects of gestation and lactation on vitamin D receptor amounts in goats and sheep. *Domestic Animal Endocrinology* **33**, 190-202.

- Liu, S, Masters, D, Ferguson, M, Thompson, A (2014) Vitamin E status and reproduction in sheep: potential implications for Australian sheep production. *Animal Production Science* **54**, 694-714.
- Madsen, F, Lentz, D, Miller, J, Hansard, S (1976) Effect of glucagon infusion on plasma magnesium, glucose, and insulin in bull calves. *Journal of Dairy Science* **59**, 1599-1602.
- Majeed, AF, Taha, MB (1989) Preliminary study on treatment of ringwomb in Iraqi goats. *Animal Reproduction Science* **18**, 199-203.
- Makoni, M, Eckert, J, Anne Pereira, H, Nizet, V, & Lawrence, SM (2016) Alterations in neonatal neutrophil function attributable to increased immature forms. *Early Human Development*, **103**, 1-7. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0378378215301900>
- Mangelsdorf, DJ, Ong, ES, Dyck, JA, Evans, RM (1990) A nuclear receptor that identifies a novel retinoic acid response pathway. *Nature* **345**, 224.
- Marret, S, Doyle, LW, Crowther, CA, Middleton, P (2007) Antenatal magnesium sulphate neuroprotection in the preterm infant. *Seminars in Fetal and Neonatal Medicine* **12**, 311-317.
- Martin, P, Bateson, PPG., Bateson, P (2007) Measuring behaviour: an introductory guide. Cambridge University Press.
- Masters DG, Hancock S, Refshauge G, Robertson S, Bhanugopan M, Friend M, Thompson AN (2017). Mineral status of reproducing ewes grazing vegetative cereal crops. *Animal Production Science* DOI 10.1071/AN16530.
- Masters, DG, Hancock, S, Refshauge, G, Robertson, S, McGrath, S, Bhanupogan, M, Friend, M, Thompson, AN (2018). Mineral supplements improve calcium status of pregnant ewes grazing vegetative cereal crops. *Animal Production Science*. Online.
- Martens, H, Schweigel, M (2000) Pathophysiology of grass tetany and other hypomagnesemias: implications for clinical management. *Veterinary Clinics of North America: Food Animal Practice* **16**, 339-368.
- Martin, TJ, Udagawa, N (1988) Hormonal regulation of osteoclast function. *Trends in Endocrinology and metabolism* **9**, 6-12.
- Martinez, N, Risco, CA, Lima, FS, Bisinotto, RS, Greco, LF, Ribeiro, ES, Maunsell, F, Galvão, K, Santos, JEP (2012) Evaluation of periparturient calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. *Journal of Dairy Science* **95**, 7158-7172.
- Martinez, N, Sinedino, LDP, Bisinotto, RS, Ribeiro, ES, Gomes, GC, Lima, FS, Greco, LF, Risco, CA, Galvão, KN, Taylor-Rodriguez, D, Driver, JP, Thatcher, WW, Santos, JEP (2014) Effect of induced sub-clinical hypocalcemia on physiological responses and neutrophil function in dairy cows. *Journal of Dairy Science* **97**, 874-887.
- Masters, DG, Fels, HE (1980) Effect of zinc supplementation on the reproductive performance of grazing Merino ewes. *Biological Trace Element Research* **2**, 281-290.
- Masters, DG, Thompson, AN (2016) Grazing crops: implications for reproducing sheep. *Animal Production Science* **56**, 655-668.

- Matsunobu, S, Terashima, Y, Senshu, T, Sano, H, Itoh, H (1990) Insulin secretion and glucose uptake in hypomagnesemic sheep fed a low magnesium, high potassium diet. *The Journal of Nutritional Biochemistry* **1**, 167-171.
- Mavrogianni, VS, Brozos, C (2008) Reflections on the causes and the diagnosis of peri-parturient losses of ewes. *Small Ruminant Research* **76**, 77-82.
- Mayer, GP, Ramberg, CF, Kronfeld, DS, Buckle, R, Sherwood, L, Aurbach, G, Potts, J (1969) Plasma Parathyroid hormone concentration in hypocalcaemic parturient Cows. *American Journal of Veterinary Research in Veterinary Science* **30**, 1587-1597.
- Mayland, HF, Grunes, DL, Lazar, VA (1976) Grass tetany hazard of cereal forages based on chemical composition. *Agronomy Journal* **68**, 665-667.
- McClure, S (2004) Mineral nutrition and its effects on gastrointestinal immune function of sheep. *Animal Production Science*, **43**, 1455-1461.
- McDonald, JW (1966) Variation in perinatal mortality of lambs with age and parity of ewes. *Proceedings of the Australian Society of Animal Production* **6**, 60-62.
- McDonald, P, Edwards, RA, Greenhalgh, JFD, Morgan, CA, Sinclair, LA, Wilkinson, RG (2011) 'Animal Nutrition.' (Pearson Education Ltd.: Harlow, Essex, England)
- McFadzean, I, Gibson, A (2002) The developing relationship between receptor-operated and store-operated calcium channels in smooth muscle. *British Journal of Pharmacology* **135**, 1-13.
- McFarlane, D (1955) Neonatal lamb mortality in the Gisborne area. *Proceedings of the New Zealand Society of Animal Production* **15**, 104-119.
- McGarry, J, Wright, P, Foster, D (1975) Hormonal control of ketogenesis. Rapid activation of hepatic ketogenic capacity in fed rats by anti-insulin serum and glucagon. *Journal of Clinical Investigation* **55**, 1202.
- McGrath, SR, Bhanugopan, MS, Dove, H, Clayton, EH, Virgona, JM, Friend, MA (2015) Mineral supplementation of lambing ewes grazing dual-purpose wheat. *Animal Production Science* **55**, 526-534.
- McGrath, SR, Lievaart, JJ, Friend, MA (2013) Extent of utilisation of dual-purpose wheat for grazing by late-pregnant and lambing ewes and producer-reported incidence of health issues in southern New South Wales. *Australian Veterinary Journal* **91**, 432-436.
- McHugh, N, Berry, DP, Pabiou, T (2016) Risk factors associated with lambing traits. *Animal* **10**, 89-95
- McNeill, DM, Roche, JR, Stockdale, CR, McLachlan, BP (2002) Nutritional strategies for the prevention of hypocalcaemia at calving for dairy cows in pasture-based systems. *Australian Journal of Agricultural Research* **53**, 755-770.

- McSporran, KD (1975) Some aspects of dystocia in sheep with particular reference to Romney stud ewes. Massey University.
- McSporran, KD, Fielden, ED (1979) Studies on dystocia in sheep II: Pelvic measurements of ewes with histories of dystocia and eutocia. *New Zealand Veterinary Journal* **27**, 75-78.
- Mellado, M, Valdez, R, Lara, L, Garcia, J (2004) Risk factors involved in conception, abortion, and kidding rates of goats under extensive conditions. *Small Ruminant Research* **55**, 191-198.
- Miller, J, Madsen, F, Lentz, D, Wong, W-O, Ramsey, N, Tysinger, C, Hansard, S (1980) Blood plasma magnesium, potassium, glucose, and immunoreactive insulin changes in cows moved abruptly from barn feeding to early spring pasture. *Journal of Dairy Science* **63**, 1073-1079.
- Mishra, OP, Delivoria-Papadopoulos, M (1999) Cellular mechanisms of hypoxic injury in the developing brain. *Brain Research Bulletin* **48**, 233-238.
- Moate, PJ, Schneider, KM, Leaver, DD, Morris, DC (1987) Effect of 1,25 dihydroxyvitamin D3 on the calcium and magnesium metabolism of lactating cows. *Australian Veterinary Journal* **64**, 73-75.
- Morgan, JE, Fogarty, NM, Nielsen, S, Gilmour, AR (2007) The relationship of lamb growth from birth to weaning and the milk production of their primiparous crossbred dams. *Australian Journal of Experimental Agriculture* **47**, 899-904.
- Mukasa-Mugerwa, E, Said, AN, Lahlou-Kassi, A, Sherington, J, Mutiga, ER (1994) Birth weight as a risk factor for perinatal lamb mortality, and the effects of stage of pregnant ewe supplementation and gestation weight gain in Ethiopian Menz sheep. *Preventive Veterinary Medicine* **19**, 45-56.
- Mulligan, F, O Grady, L, Rice, D, Doherty, M (2006) Production diseases of the transition cow: Milk fever and sub-clinical hypocalcaemia. *Irish Veterinary Journal* **59**, 697.
- National Research Council (2005) 'Mineral Tolerance of Animals.' (The National Academies Press: Washington)
- Nawito, MF, Hameed, ARAE, Sosa, ASA, Mahmoud, KGM (2016) Impact of pregnancy and nutrition on oxidant/antioxidant balance in sheep and goats reared in South Sinai, Egypt. *Veterinary World* **9**, 801-805.
- Newton, GL, Tucker, RE, Fontenot, JP, Polan, CE (1972) Effects of high dietary potassium intake on metabolism of magnesium by sheep. *Journal of Animal Science* **35**, 440.
- Nosdøl, G, Waage, S (1981) Hypocalcaemia in the ewe. *Nordisk Veterinaermedicin* **33**, 310-326.
- Nowak, R, Poindron, P (2006) From birth to colostrum: early steps leading to lamb survival. *Reproduction, Nutrition and Development* **46**, 431-446.
- Oetzel, G, Olson, J, Curtis, C, Fettman, M (1988) Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. *Journal of Dairy Science* **71**, 3302-3309.
- Oldham, CM, Thompson, AN, Ferguson, MB, Gordon, DJ, Kearney, GA, Paganoni, BL (2011) The birthweight and survival of Merino lambs can be predicted from the profile of liveweight change of their mothers during pregnancy. *Animal Production Science* **51**, 776-783.

- Paganoni, B, Ferguson, M, Kearney, G, Thompson, A (2014) Increasing weight gain during pregnancy results in similar increases in lamb birthweights and weaning weights in Merino and non-Merino ewes regardless of sire type. *Animal Production Science* **54**, 727-735.
- Paynter, DI (1996) Diagnosis of mineral deficiencies. In 'Detection and Treatment of Mineral Disorders in Grazing Sheep.' (Eds DG Masters, CL White.) pp. 45-56. (Australian Centre for International Agricultural Research: Canberra, Australia)
- Perlman, JM (2006) Intervention strategies for neonatal hypoxic-ischemic cerebral injury. *Clinical Therapeutics* **28**, 1353-1365.
- Phillips, C, Mohamed, M, Chiy, P (2006) The critical dietary potassium concentration for induction of mineral disorders in non-lactating Welsh Mountain sheep. *Small Ruminant Research* **63**, 32-38.
- Phillips, WA, Horn, GW (2008) Intake and digestion of wheat forage by stocker calves and lambs. *Journal of Animal Science* **86**, 2424-2429.
- Phillipson, A, Storry, J (1965) The absorption of calcium and magnesium from the rumen and small intestine of the sheep. *The Journal of Physiology* **181**, 130.
- Pilny, AA (2014) *Endocrinology, An Issue of Veterinary Clinics: Exotic Animal Practice*. Philadelphia, United States: Elsevier Health Sciences.
- Plush, KJ, Brien, FD, van Wettere, WHEJ, Hynd, PI (2016) Preparing the neonate for the transition from intra-uterine to extra-uterine life. *Proceedings of the 31st Biennial Conference of the Australian Society of Animal Production* Ref No. 1415. Available at http://www.asap.asn.au/wp-content/uploads/abstract-2015/302/attach_brief.pdf [Accessed July 4 -7].
- Ram, L, Schonewille, JT, Martens, H, Van't Klooster, AT, Beynen, AC (1998) Magnesium absorption by wethers fed potassium bicarbonate in combination with different dietary magnesium concentrations. *Journal of Dairy Science* **81**, 2485-2492.
- Ramberg, C, GP., M, Kronfeld DS, LM., S, JT, P, GD, A (1967) Plasma calcium and parathyroid hormone responses to EDTA infusion in the cow. *American Journal of Physiology* **213**, 878-882.
- Ramberg, C, Johnson, E, Fargo, R, Kronfeld, D (1984) Calcium homeostasis in cows, with special reference to parturient hypocalcemia. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **246**, R698-R704.
- Ramberg, CF, Mayer, GP, Kronfeld, DS, Phang, JM, Berman, M (1970) Calcium kinetics in cows during late pregnancy, parturition and early lactation. *American Journal of Physiology* **219**, 1166-1177.
- Rasmussen, H, Fontaine, O, Max, EE, Goodman, DBP (1979) The effect of 1 alpha -hydroxyvitamin D3 administration on calcium transport in chick intestine brush border membrane vesicles. *Journal of Biological Chemistry* **254**, 2993-2999.

- Rayssiguier, Y (1977) Hypomagnesemia resulting from adrenaline infusion in ewes: its relation to lipolysis. *Hormone and Metabolic Research* **9**, 309-314.
- Reeve, J, Sharkey, M (1980) Effect of stocking rate, time of lambing and inclusion of lucerne on prime lamb production in north-east Victoria. *Australian Journal of Experimental Agriculture* **20**, 637-653.
- Refshauge, G, Brien, FD, Hinch, GN, van de Ven, R (2016) Neonatal lamb mortality: factors associated with the death of Australian lambs. *Animal Production Science* **56**, 726-735.
- Reinhardt, TA, Lippolis, JD, McCluskey, BJ, Goff, JP, Horst, RL (2011) Prevalence of sub-clinical hypocalcemia in dairy herds. *The Veterinary Journal* **188**, 122-124.
- Risco, CA, Reynolds, JP, Hird, D (1984) Uterine prolapse and hypocalcemia in dairy cows. *Journal of the American Veterinary Medical Association* **185**, 1517-1519.
- Robertson, SM, Friend, MA, King, BJ (2008) Mild congenital goitre increases lamb mortality in southern New South Wales. *Australian Journal of Experimental Agriculture* **48**, 995-998.
- Robinson, JJ, Rooke, JA, McEvoy, TG (2002) Nutrition for conception and pregnancy. In 'Sheep Nutrition.' (Eds M Freer, H Dove.) pp. 189-211. (CSIRO Publishing: Collingwood, Australia)
- Roche, J, Dalley, D, Moate, P, Grainger, C, Rath, M, O'mara, F (2003) Dietary cation-anion difference and the health and production of pasture-fed dairy cows 2. Nonlactating periparturient cows. *Journal of Dairy Science* **86**, 979-987.
- Rodan, GA, Martin, TJ (1981) Role of osteoblasts in hormonal-control of bone-resorption - a hypothesis. *Calcified Tissue International* **33**, 349-351.
- Romani, A, Scarpa, A (1990) Hormonal control of Mg²⁺ transport in the heart. *Nature* **346**, 841-844.
- Sansom, BF, Bunch, KJ, Dew, SM (1982) Changes in plasma calcium, magnesium, phosphorus and hydroxyproline concentrations in ewes from twelve weeks before until three weeks after lambing. *British Veterinary Journal* **138**, 393-401.
- Sargison, N (2009) 'Sheep Flock Health: A Planned Approach ' (Wiley: Chichester)
- Schachter, D, Dowdle, EB, Schenker, H (1960) Accumulation of Ca-45 by slices of the small intestine. *American Journal of Physiology* **198**, 275-279.
- Schlumbohm, C, Harmeyer, J (2003) Hypocalcemia reduces endogenous glucose production in hyperketonemic sheep. *Journal of Dairy Science* **86**, 1953-1962.
- Schlumbohm, C, Harmeyer, J (2008) Twin-pregnancy increases susceptibility of ewes to hypoglycaemic stress and pregnancy toxaemia. *Research in Veterinary Science* **84**, 286-299.
- Schneider, KM, Parkinson, GB, Houston, JC, Leaver, DD (1985) 1,25 dihydroxyvitamin D₃ increases plasma magnesium and calcium in sheep fed liquid diets low in calcium and magnesium. *Australian Veterinary Journal* **62**, 82-85.
- Schonewille, JT, Beynen, AC, Klooster, ATvt, Wouterse, H, Ram, L (1999a) Dietary potassium bicarbonate and Ppotassium citrate have a greater inhibitory effect than does potassium chloride on magnesium absorption

in wethers. *Journal of Nutrition* **129**, 2043-2047. Available at <http://jn.nutrition.org/cgi/content/abstract/129/11/2043> [Accessed November 1, 1999].

- Schonewille, JT, Van't Klooster, AT, Wouterse, H, Beynen, A (1999b) Hypocalcemia induced by intravenous administration of disodium ethylenediaminetetraacetate and its effects on excretion of calcium in urine of cows fed a high chloride diet. *Journal of Dairy Science* **82**, 1317-1324.
- Schreurs, NM, Kenyon, PR, Mulvaney, FJ, Morel, PCH, West, DM, Morris, ST (2010) Effect of birthweight and birth rank on the survival of single and twin lambs born to ewe lambs. *Animal Production Science* **50**, 460-464.
- Schuster, NH, Watts, HJ, Webster, ME, Campbell, RW (1969) Experimental grass tetany in the ewe. *Australian Veterinary Journal* **45**, 508-516.
- Scott, P (2013) Hypocalcaemia in ewes. *Livestock* **18**, 37-40.
- Selvamurugan, N, Chou, WY, Pearman, AT, Pulumati, MR, Partridge, NC (1998) Parathyroid hormone regulates the rat collagenase-3 promoter in osteoblastic cells through the cooperative interaction of the activator protein-1 site and the runt domain binding sequence. *Journal of Biological Chemistry* **273**, 10647-10657.
- Sentinel Flock Project DEPI (2012) Sentinel flock project. . Victorian Department of Environment and Primary Industries. Unpublished data.
- Shaker, JL, Deftos, L (2016) Calcium and phosphate homeostasis. Disease of bone and mineral metabolism.(Ed. F Singer.) Available at <http://www.endotext.org/section/parathyroid>. [Accessed December 2016].
- Silva, R, Noakes, D (1984) The effect of experimentally induced hypocalcaemia on uterine activity at parturition in the ewe. *Theriogenology* **21**, 607-623.
- Smith, BSW, Wright, H (1984) Relative contributions of diet and sunshine to the overall vitamin D status of the grazing ewe. *Veterinary Record* **115**, 537-538.
- Smith, C, Wagner, P (1985) Electrolyte imbalances and metabolic disturbances in endurance horses. *Compendium on Continuing Education for the Practicing Veterinarian (USA)* **7**, 575-585.
- Smith, GM (1977) Factors affecting birth weight, dystocia and preweaning survival in sheep. *Journal of Animal Science* **44**, 745-753.
- Smith, KF, Rebetzke, GJ, Eagles, HA, Anderson, MW, Easton, HS (1999) Genetic control of mineral concentration and yield in perennial ryegrass (*Lolium perenne* L.), with special emphasis on minerals related to grass tetany. *Australian Journal of Agricultural Research* **50**, 79-86.
- Snowder, GD, Glimp, HA (1991) Influence of breed, number of suckling lambs, and stage of lactation on ewe milk production and lamb growth under range conditions. *Journal of Animal Science* **69**, 923-30.

- Sobiraj, A, Busse, G, Gips, H, Bostedt, H (1986) Investigations into the blood plasma profiles of electrolytes, 17 β -oestradiol and progesterone in sheep suffering from vaginal inversion and prolapse ante partum. *British Veterinary Journal* **142**, 218-223.
- Stewart, PA (1983) Modern quantitative acid-base chemistry. *Canadian Journal of Physiology and Pharmacology* **61**, 1444-1461.
- Stubbings, D (1971) Observations on serum calcium levels in ewes in North Lincolnshire in relation to prolapse of the vagina and incomplete cervical dilatation. *Veterinary Record* **89**, 296-300.
- Suarez, A, Pulido, N, Casla, A, Casanova, B, Arrieta, F, Rovira, A (1995) Impaired tyrosine-kinase activity of muscle insulin receptors from hypomagnesaemic rats. *Diabetologia* **38**, 1262-1270.
- Suki, WN, Massry, SG (2012) 'Therapy of Renal Diseases and Related Disorders.' (Springer Science and Business Media: New York)
- Suttle, NF (2010) 'Mineral Nutrition of Livestock.' (CABI Publishing: Wallingford)
- Suttle, NF, Field, AC (1969) Studies on magnesium in ruminant nutrition. *British Journal of Nutrition* **23**, 81-90.
- Swerczek, TW (2008) Nitrate toxicity, sodium deficiency and the grass tetany syndrome. *The Growers Solution* **21**, 3. Available at www.growersmineral.com [Accessed March 1, 2010].
- Sykes, AR (2007) Deficiency of mineral macro-elements. In 'Diseases of Sheep, 4th edn.' (Ed. ID Aitken.) (Blackwell Publishing: Oxford, UK)
- Tabatabaei, S, Nikbakht, G, Vatankhah, M, Sharifi, H, Alidadi, N (2013) Variation in colostral immunoglobulin G concentration in fat tailed sheep and evaluation of methods for estimation of colostral immunoglobulin content. *Acta Veterinaria Brno* **82**, 271-275.
- Takagi, H, Block, E (1991a) Effects of manipulating dietary cation-anion balance on macromineral balance in sheep. *Journal of Dairy Science* **74**, 4202-4214.
- Takagi, H, Block, E (1991b) Effects of various dietary cation-anion balances on response to experimentally induced hypocalcemia in sheep. *Journal of Dairy Science* **74**, 4215-4224.
- Terashima, Y, Muzushima, Y, Yamaoka, R, Itoh, H (1983) Effects of intravenous infusion of noradrenaline on plasma levels of Ca, inorganic P and Mg in sheep fed high or low calcium diet. *Japanese Journal of Zootechnical Science (Japan)*
- Terashima, Y, Tucker, RE, Deetz, LE, Degregorio, RM, Muntifering, RB, Mitchell Jr, GE (1982) Plasma magnesium levels as influenced by cold exposure in fed or fasted sheep. *The Journal of Nutrition* **112**, 1914-1920.
- Thompson, JK, Reid, RL (1981) Mineral status of beef cows and sheep on spring pasture fertilized with kieserite. *Journal of Animal Science* **52**, 969-976.
- Trawńska, B, Polonis, A, Lechowski, J, Tymczyna, L, Borowski, R, Gizińska, K, 2013. Effect of the addition of magnesium salt to a feed mixture on intestinal microflora, health, and production of sows. *Bulletin of the Veterinary Institute in Pulawy*. **57**, 69.
- Treacher, TT, Caja, G (2002) Nutrition during lactation. In 'Sheep Nutrition.' (Eds M Freer, H Dove.) pp. 213-236. (CAB International: Wallingford, UK)

- Tribe, RM (2001) Regulation of human myometrial contractility during pregnancy and labour : are calcium homeostatic pathways important? *Experimental Physiology* **86**, 247-254.
- Unanian, M, Feliciano Silva, A (1984) Trace elements deficiency: association with early abortion in goats. *International Goat and Sheep Research* **2**, 129-34.
- Underwood, EJ, Suttle, NF (1999) 'The Mineral Nutrition of Livestock.' (CAB International: Wallingford)
- Vagg, M, Payne, J (1970) The effect of ammonium chloride induced acidosis on calcium metabolism in ruminants. *British Veterinary Journal* **126**, 531-537.
- van den Bergh, WM (2005) Magnesium sulfate in aneurysmal subarachnoid hemorrhage - a randomized controlled trial. *Stroke* **36**, 1011-1015.
- Vannucci, RC, Perlman, JM (1997) Interventions for perinatal hypoxic-ischemic encephalopathy. *Pediatrics* **100**, 1004-1114.
- Venu, L, Kishore, YD, Raghunath, M (2005) Maternal and perinatal magnesium restriction predisposes rat pups to insulin resistance and glucose intolerance. *The Journal of Nutrition* **135**, 1353-1358.
- Vernon, R, Faulkner, A, Hay, W, Calvert, D, Flint, D (1990) Insulin resistance of hind-limb tissues in vivo in lactating sheep. *Biochemical Journal* **270**, 783-786.
- Waage, S, Wangenstein, G (2013) Short-term and long-term outcomes of ewes and their offspring after elective cesarean section. *Theriogenology* **79**, 486-494.
- Waghorn, GC, Burke, JL, Kolver, ES (2007) Principles of feeding value. In 'Pasture and Supplements for Grazing Animals.' (Eds PV Rattray, IM Brookes, AM Nicol.) Vol. Occasional Publication No. 14 pp. 26. (New Zealand Society of Animal Production: Hamilton, New Zealand)
- Walkden-Brown, SW, Kahn, LP (2002) Nutritional modulation of resistance and resilience to gastrointestinal nematode infection - a review. *Asian-Australasian Journal of Animal Science* **15**, 912-924.
- Ward, TL, Valberg, SJ, Gallant, EM, Mickelson, JR (2000) Calcium regulation by skeletal muscle membranes of horses with recurrent exertional rhabdomyolysis. *American Journal of Veterinary Research* **61**, 242-247.
- Wasserman, R, Taylor, A (1969) Some aspects of the intestinal absorption of calcium, with special reference to vitamin D. *Mineral Metabolism* **3**, 321-403.
- Wassermann, RH, Brindak, ME, Meyer, SA, Fullmer, CS (1982) Evidence for multiple effects of vitamin D3 on calcium absorption: response of rachitic chicks, with or without partial vitamin D3 repletion, to 1,25-dihydroxyvitamin D3. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 7939-7943.

- Watson, R, Alexander, G, Cumming, I, McDonald, J, McLaughlin, J, Rizzoli, D, Williams, D (1968) Reduction of perinatal loss of lambs in winter in western Victoria by lambing in sheltered individual pens. *Proceedings of the Australian Society of Animal Production* **7**, 243-249.
- Watt, B (2006) Hypocalcaemia in lactating drought fed ewes supplemented with recommended levels of calcium. *Australian Society of Animal Production 26th Biennial Conference 2006* Short Communication number 10. Available at <http://www.asap.asn.au/livestocklibrary/2006/SC10-watt.pdf>
- Weekes, T, Sasaki, Y, Tsuda, T (1983) Enhanced responsiveness to insulin in sheep exposed to cold. *American Journal of Physiology-Endocrinology and Metabolism* **244**, E335-E345.
- Weston, RH (2002) Constraints on feed intake by grazing sheep In 'Sheep Nutrition.' (Eds M Freer, H Dove.) pp. 27-49.
- Wilkens, MR, Praechter, C, Breves, G, Schröder, B (2016) Stimulating effects of a diet negative in dietary cation-anion difference on calcium absorption from the rumen in sheep. *Journal of Animal Physiology and Animal Nutrition* **100**, 156-166.
- Wilkens, MR, Richter, J, Fraser, DR, Liesegang, A, Breves, G, Schröder, B (2012) In contrast to sheep, goats adapt to dietary calcium restriction by increasing intestinal absorption of calcium. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **163**, 396-406.
- Wilson, GF (1978) Effect of water content of Tama ryegrass on voluntary intake of sheep. *New Zealand Journal of Experimental Agriculture* **6**, 53-54.
- Wray, S, Arrowsmith, S (2012) *Fundamental Biology and Mechanisms of Disease*. Elsevier Science & Technology, ProQuest Ebook Central.
- Wylie, MJ, Fontenot, JP, Greene, LW (1985) Absorption of magnesium and other macrominerals in sheep infused with potassium in different parts of the digestive tract. *Journal of Animal Science* **61**, 1219-1229.
- Yano, H, Nagoaka, C, Kawashima, R (1984) Effects of intravenous glucose or epinephrine injection on plasma concentrations of potassium, magnesium and insulin in sheep. *Journal of Nutritional Science and Vitaminology* **30**, 535-540.
- Zaborski, D, Grzesiak, W, Szatkowska, I, Dybus, A, Muszynska, M, Jedrzejczak, M (2009) Factors affecting dystocia in cattle. *Reproduction in Domestic Animals* **44**, 540-551.
- Zang, J, Chen, J, Tian, J, Wang, A, Liu, H, Hu, S, Che, X, Ma, Y, Wang, J, Wang, C, Du, G, Ma, X (2014) Effects of magnesium on the performance of sows and their piglets. *Journal of Animal Science and Biotechnology* **5**, 39.
- Zemel, M (2004) Role of calcium and dairy products in energy partitioning and weight management. *American Journal of Clinical Nutrition* **79**, 907S-912S.

13. LIST OF ABBREVIATIONS

Ca	calcium
Cu	copper
I	iodine
K	potassium
Mg	magnesium
Na	sodium
OSi	ROM/TAC
P	phosphorus
PTH	parathyroid hormone
ROM	reactive oxygen species
Se	selenium
TAC	total plasma antioxidant capacity
Zn	zinc