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Original Article

Maternal supplementation of twin bearing ewes with calcium and magnesium alters immune status and weight gain of their lambs

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ABSTRACT

This study investigated the effect of supplementation of ewes with calcium (Ca) and magnesium (Mg) in late gestation and early lactation on the plasma mineral concentration, hormone profile and immunity responses of ewes and their lambs. Twin bearing ewes were allocated between four dietary treatment groups (n = 11 per treatment): control (0.33% DM Ca and 0.28% DM Mg); high Ca (0.72% DM Ca and 0.28% DM Mg); high Mg (0.33% DM Ca, 0.48% DM Mg); and high Ca + Mg (0.66% DM Ca and 0.47% DM Mg), offered as part of a whole ration. Ewes were fed the treatment supplement from around one month prior to lambing to one month after lactation. Blood and urine samples were collected at seven time-points: weekly starting at 5 weeks prior to lambing; within 12 h post lambing (+12 h); and then at fortnightly intervals at 2 week (+2 W) and at 4 week (+4 W) post lambing. Colostrum/milk samples from ewes and blood samples from lambs were collected at +12 h, +2 W and +4 W. Live weight of lambs were measured at +12 h, +2 W and +4 W.

The plasma concentration of PTH, $1,25(OH)_2D_3$ and $25(OH)D_3$ was lowest at +2 W ($P \leq 0.002$). Ewes from the Ca + Mg group had the lowest mean concentration of $1,25(OH)_2D_3$ than the other groups ($P = 0.005$). Magnesium supplementation improved the plasma Mg concentration over time in ewes ($P < 0.001$) and lambs from the control group had lower plasma Mg concentration compared to the treatment groups at +4 W ($P = 0.001$). Oxidative burst response in lambs supplemented with Ca tended to be greater at +4 W than the other groups at the same time point ($P = 0.051$) and Mg supplementation increased total antioxidant capacity (TAC) concentration in lambs ($P = 0.040$). The average daily weight gain of lambs was 204 g/lamb/d for the Ca group, 207 g/lamb/d for the Mg group, 245 g/lamb/d for the Ca + Mg group which were greater than the control group (148 g/lamb/d) ($P < 0.001$).

Despite the normal concentration of Ca and Mg in the plasma, supplementation of ewes with Ca and Mg from one month prior to lambing to one month post lambing improved TAC concentration and weight gain in lambs.

Introduction

Calcium (Ca) and magnesium (Mg) are vital for a number of physiological processes such as boosting the immune system, regulating energy metabolism, bone structure and muscle contraction and also the requirements of pregnant and lactating ewes for these macro-minerals is high (McClure, 2004). Engagement of pathogens to receptors on leukocyte cells triggers the release of Ca from endoplasmic reticulum (ER) to induce oxidative burst in leukocyte cells and digest engulfed pathogen (Allard, Long, Block & Zhao, 1999; Ducusin et al., 2003). The role of Mg in the immune system is through activation of the adaptive immune system, acting as a cofactor for immunoglobulin synthesis, immune cell adherence, C3 convertase (complement system), antibody dependent catalysis, and T-helper and B-cell adherence (Feske, 2007; Laires & Monteiro, 2008).

Ewes have high requirements for Ca and Mg at pregnancy and lactation because of fetus growth and milk production. The growing fetus and neonatal ruminant are reliant on their mother for nutrients like glucose and minerals to obtain optimal growth rate (Boland et al., 2016; Dang et al., 2013; Hernández-Castellano, Argüello, Almeida, Castro & Bendixen, 2015; Tygesen et al., 2008). The concentration of Ca and Mg in ewe's colostrum/milk is high and is almost double that

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Table 1
Proximate and mineral analysis of pellets offered to ewes during late gestation and early lactation (Mean ± S.E).

Feed contents	Treatment – Mean ± SE					
	Control	Calcium	Magnesium	Calcium + Magnesium	SE	Ewe's Req[a]
DM (%)	89.5	90.9	90.6	90.7	0.39	–
NDF (%)	33.0	34.3	33.5	33.5	1.01	–
ADF (%)	15.8	16.8	15.3	15.5	0.48	–
CP (%)	14.7	14.4	14.4	14.4	0.15	–
ME (MJ/Kg DM)	12.4	12.1	12.1	12.1	0.17	–
CF (%)	7.5	8.5	7.7	9.1	0.27	–
Ca (%)	0.33	0.72	0.33	0.66	0.02	>0.4%
Mg (%)	0.28	0.29	0.43	0.47	0.007	>0.09%
K (%)	0.72	0.73	0.79	0.81	0.006	0.5–3%
Na (%)	0.20	0.21	0.20	0.23	0.009	>0.09%
P (%)	0.36	0.34	0.43	0.43	0.012	>0.2

DM: Dry Matter, **NDF:** Neutral Detergent fiber, **ADF:** Acid Detergent fiber, **CP:** Crude Protein,.
ME: Metabolised Energy, **CF**: Crude fiber.
[a] Derived from Freer et al. (2007) and Masters et al. (2018).

observed in cow's milk (Thirunavukkarasu, Kathiravan, Kalaikannan & Jebarani, 2010). High drainage of Ca and Mg from plasma for fetal growth and colostrum and milk synthesis normally exceeds the available plasma pool by a factor of several times, which consequently induces severe metabolic stress (Brozos, Mavrogianni & Fthenakis, 2011; Campion et al., 2016; Kenyon et al., 2009). Metabolic stress causes immune suppression in ewes which results in an increase in production of reactive oxygen metabolites (ROS), impaired neutrophil function (chemotaxis, phagocytosis and oxidative burst), a decrease in antibody response and cytokine production (Anugu, Petersson-Wolfe, Combs & Petersson, 2013). Cows with hypocalcaemia suffer more from immune suppression due to high cortisol concentration, reduced blood neutrophil counts and impaired neutrophil function (Bicalho et al., 2014; Martinez et al., 2012). Mastectomised cows do not develop hypocalcaemia and do not suffer from the same degree of immune suppression as milk producing cows (Nonnecke, Kimura, Goff & Kehrli, 2003), reiterating the role of Ca in the immune system. Immunosuppression due to the increased mineral and energy demands and high stress at parturition and lactation increases the ewe's susceptibility to develop metabolic disorders and could lead to reproductive wastage such as abortion or neonatal death (Bernabucci, Ronchi, Lacetera & Nardone, 2005).

Therefore, it is important to understand the effect of Ca and Mg supplementation during late gestation, transition and early lactation on immune function in ewes and their lambs for better management of ewes and their newborns during critical periods. The objectives of this study were to identify the effect of Ca and Mg supplementation from one month before lambing to one month after lambing on the energy regulation and immune response of ewes and their lambs, and also on the growth of newborn lambs. We hypothesised that maternal supplementation of ewes with Ca and Mg above their requirements at gestation and lactation would improve the immune system and production of ewes and their lambs.

Materials and methods

The study was conducted at Charles Sturt University, Wagga Wagga, NSW, Australia from April 2017 to August 2017. The study was approved by the Charles Sturt University Animal Care and Ethics Committee (protocol number A16077).

Sheep management and measurements

Ewes were naturally joined to rams for five weeks. A mob of twin-bearing Merino ewes (n = 44; 3–5 years old) at day 116 of after the commencement of joining were weighed (mean live weight 60.7 ± 0.3 kg) and condition scored (mean body condition score (BCS) 2.6 ± 0.3 (scale 1–5) (Jefferies, 1961)) on *date*. Ewes were blocked by BCS and randomly allocated to individual pens (area 9 m^2 per pen) in an outdoor feeding facility. Ewes were fed with customised pellets (Quayle Milling Pty. Ltd. Yass, NSW, Australia) and the content of Ca and Mg in the pellets were different based on the treatment group. Pellets were made using base components that include barley, lupins, oat hulls, bentonite, millmix and canola. The Ca and Mg content of the pellets were manipulated by addition of ground limestone and/or magnesium oxide. Ewes at day 116 after the commencement of joining were randomly allocated to one of four treatments based on the Ca and Mg content of the pellets by which they were fed: Control group (0.33% DM Ca and 0.28% DM Mg), high Ca (0.72% DM Ca and 0.28% DM Mg); high Mg (0.33% DM Ca, 0.48% DM Mg); and high Ca + Mg (0.66% DM Ca and 0.47% DM Mg), offered as part of a whole ration. The Ca and Mg levels in the Control pellet were formulated to meet minimum requirements for pregnant and lactating ewes (minimum 0.4% DM and 0.09% DM for Ca and Mg respectively, (Freer, Dove & Nolan, 2007)). The Ca and Mg concentration in the supplemented groups was designed to be above animal requirements. Ewes were randomly allocated to one of four treatments. The animals had free access to water at all times throughout the experimental period. The proximate and mineral content of the customised pellets offered to ewes at four treatment groups are presented in Table 1.

The animals were fed their respective pellets daily from 5 week prior to the commencement of lambing (day 120, late gestation) to 4 week post lambing (early lactation). Ewes were fed 2.5 kg DM of corresponding pellets/ewe/d during gestation and this was increased to 3 kg DM of pellets/ewe/d during lactation to meet the requirements.

Samples of pellets from each treatment were collected daily and made into composite fortnight samples and submitted for proximate analysis using near infra-red reflectance (NIR) spectroscopy by the New South Wales Department of Primary Industries Feed Quality Service (NSW DPI FQS, Wagga Wagga, New South Wales). Mineral analyses were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Varian 710-ES) at wavelengths 315.89 nm for Ca, 279.08 nm for Mg, and 213.62 nm for P at the Environmental Analysis Laboratory (EAL), Charles Sturt University, Wagga Wagga, New South Wales, Australia.

Collection of blood, urine and milk samples

Blood and urine samples were collected weekly starting at 5 week prior to lambing (−5 W, −4 W, −3 W, −2 W, −1 W), 12 h post lambing (+12 h) and then at 2 weeks (+2 W) and 4 weeks (+4 W) post lambing. Colostrum samples from ewes were collected at +12 h, and milk samples were collected at +2 W and +4 W and stored at −30 °C for further analysis. Blood samples from lambs were collected

within first 12 h of birth (+12 h) and at +2 W and +4 W. Blood samples were collected from the jugular vein using 18 G needle into heparinised vacutainer tubes (Greiner bio-one, #455084) and immediately placed on ice. Blood samples were centrifuged within 5 h of sampling at 2000 × g at 4 °C for 20 min. Plasma was removed and stored at −30 °C for further analysis. The remaining blood was used for isolation of leukocytes. Live weight of lambs were recorded at same time point at which blood samples were collected.

From the ewes, urine samples were collected by the nasal occlusion method (Hoogendoorn, Betteridge, Costall & Ledgard, 2010) and milk samples by hand milking were collected into 50 ml vials and then aliquoted into 5 ml sample tubes and stored at −30 °C for further analysis.

Calcium, magnesium and phosphorus concentration in plasma, urine and milk

A solution of 1% nitric acid was used to process and dilute plasma, urine and milk samples (dilution 1:10). Samples were subsequently analysed for the concentration of Ca, Mg and P using ICP-AES (Varian 710-ES) at EAL, Charles Sturt University, Wagga Wagga, New South Wales, Australia.

Urine creatinine was measured quantitatively by a kinetic color test on Beckman coulter auto-analyser at the Veterinary Diagnostic Laboratory (VDL), Charles Sturt University. Creatinine is excreted in the urine at a constant rate within 24 h (Wills, 1969); thus, urine creatinine in our study was measured to report the concentration of urinary Ca, Mg and P as a ratio to urinary creatinine. The excretion of minerals in urine is represented as urine Ca: creatinine ratio, urine Mg: creatinine ratio and urine P: creatinine ratio.

Parathyroid hormone, 25 hydroxy vitamin D_3 and 1,25 di hydroxy vitamin D_3 assays

Plasma concentration of parathyroid hormone (PTH), 1,25 dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) and 25 hydroxy vitamin D_3 ($25OHD_3$) were measured by the enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, # MBS109006; Cusabio, # MBS017166; Cusabio, # MBS046451, respectively, Houston, USA).

Determination of phagocytosis and oxidative burst response of leukocytes

Leukocyte cells were isolated from the blood sample after plasma removal. The erythrocytes were separated and lysed by adding an erythrocyte digest buffer. After centrifugation at 400 × *g* for 5 min, the isolated leukocyte cells were resuspended in phosphate buffered saline (PBS) (# P4417, Sigma Aldrich, Saint Laius, Missouri, USA) and counted in a hemocytometer chamber (Abcam).

Cayman's phagocytosis assay kit (IgG FITC) (Cayman Chemical, Michigan, USA, # 500290) was used to measure phagocytic function of leukocyte cells. The engulfed fluorescence beads were detected using Gallios Flow Cytometer (# 773231AD, Beckman Coulter, Georgia, USA,).

For the measurement of oxidative burst, the remaining isolated leukocyte cells were reconstituted to a concentration of 2×10^5 cells/100 µl of medium. Then, 100 µl of leukocytes were pipetted in triplicates into a 96 well plate (# O2222381, Thermo Fisher Scientific, Waltham, USA). Negative or non-activated control for this assay was made by pipetting 100 µl of medium as triplicate. Eighty µl of phorbol myristate acetate (# P1585, Sigma Aldrich) at a final concentration of 400 nM was added to all wells. Also, 20 µl luminol was aded to all wells at final concentration of 10 µM. The plate was incubated at 37 °C and luminescence was recorded every 5 min for 2 h using FLUOstar Omega microplate reader. Production of reactive oxygen species was calculated as follows:

Oxidative burst response of leukocytes = (Sum of luminescence measurement of leukocytes over 2 h) – (Sum of luminescence measurement of negative control wells over 2 h).

Plasma oxidative status determinations and measurement of immunoglobulin G in colostrum and lamb plasma

Reactive oxygen metabolites (ROM) were quantified as an indicator of Reactive Oxygen Species (ROS) by d-ROM test (# MC 003, Diacron international, Grossetto, Italy). Results are expressed in arbitrary 'Carratelli Units' (CarrU), where 1 CarrU is equivalent to the oxidizing power of 0.08 mg H_2O_2/dL.

Total antioxidant capacity (TAC) of plasma was evaluated using the OXY-Adsorbent test (# MC435, Diacron International, Grossetto, Italy) to assess all antioxidants present in plasma. Results are expressed as µmol HClO/ml. The oxidative stress index (OSi) was calculated as a ratio of ROM ÷ TAC.

Plasma IgG concentration was measured by a sheep IgG ELISA kit (#CSB-E14400Sh, Cusabio). Colostrum IgG concentration was measured by Calokit Ovino ELISA kit (# ZE/CKO96, Zeulab, Zaragoza, Spain).

Statistical analysis

All data were analysed using IBM SPSS Statistics for Windows (Version 20.0. Armonk, New York: 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. Main effects (treatment, timepoint), and their interaction (timepoint × treatment) were fitted as fixed factors, and the random factor was pen.

The residual variance were not normally distributed for phagocytosis, ROM, plasma Mg, plasma Ca, plasma P, $1,25(OH)_2D_3$, $25OHD_3$ and PTH in ewes and Phagocytosis, OSi and live weight gain in lambs. These variables were log-transformed and back-transformed for reporting means.

When the analysis of variance (ANOVA) suggested a significant treatment, time or treatment × time effect, means were compared with Fischer's least significant difference (LSD) test.

Statistical significance was accepted at $P < 0.05$ and $0.05 \leq P \geq 0.1$ are used to show the tendencies.

Results

Mineral concentration in plasma, milk and urine in ewes

Plasma Mg concentration was greatest at +4 W which was followed by +2 W compared to the other time points ($P < 0.001$) (Table 2). Ewes supplemented with Mg (Mg and Ca+Mg groups) had greater plasma Mg concentration (3.2 ± 0 0.2 for Mg group and 2.9 ± 0.2 for Ca+Mg group) compared to the control (2.5 ± 0.2) and Ca groups (2.4 ± 0.2) ($P < 0.001$). The interaction of time × treatment was significant for plasma Mg, and was greater in the Mg and Ca+Mg groups at −4 W, −3 W, −1 W, +12 h, +2 W and +4 W compared to the Ca and control groups ($P = 0.002$) (Fig. 1). Treatment did not have a significant effect on plasma Ca concentrations and the interaction of time × treatment was not significant for plasma Ca concentration. Plasma Ca concentration in ewes was the highest at +2 W and +4 W compared to the other time points ($P < 0.001$) (Table 2). Time, treatment and their interaction had no significant effect on the plasma P concentration (Table 2).

The concentration of Ca, Mg and P in colostrum did not differ significantly between treatment groups. Calcium concentration in milk (+2 W and +4 W) was greater than colostrum (+12 h) ($P = 0.021$), while Mg and P concentration in colostrum was greater than milk ($P < 0.001$) (Table 2). Milk Ca, Mg and P concentration did not differ significantly between treatments at any time point.

Urinary Ca and P excretion was highest at post-lambing compared

Table 2
Mean calcium (Ca), magnesium (Mg) and phosphorous (P) in the ewe plasma, colostrum, milk and urine (shown as a ratio to creatinine)(Mean ± SE).

	−5 W	−4 W	−3 W	−2 W	−1 W	+12 H	+2 W	+4 W	SE	*P*-value
Plasma (mg/dL)										
Ca	9.7^{c}	$8.9^{a,b}$	$9.4^{b,c}$	8.7^{a}	9.9^{c}	$9.5^{b,c}$	10.7^{d}	12.0^{e}	0.33	<0.001
Mg	2.6^{b}	$2.4^{a,b}$	$2.9^{c,d}$	$2.5^{a,b}$	$2.7^{b,c}$	$2.8^{b,c}$	3.2^{d}	3.8^{e}	0.12	<0.001
P	8.2	7.2	7.6	7.2	7.8	8.1	9.2	8.1	0.22	n.s.
Milk/colostrum (mg/dL)										
Ca	–	–	–	–	–	149.4^{a}	165.1^{b}	169.7^{b}	5.25	0.021
Mg	–	–	–	–	–	20.5^{b}	11.5^{a}	11.0^{a}	0.82	<0.001
P	–	–	–	–	–	181.8^{b}	126.9^{a}	113.8^{a}	12.3	<0.001
Urine: Creatinine										
Ca	0.014^{a}	0.010^{a}	0.008^{a}	0.010^{a}	0.01^{a}	3.3^{b}	4.7^{d}	4.2^{c}	0.002	<0.001
Mg	$0.34^{a,b}$	0.48^{c}	0.35^{b}	$0.41^{b,c}$	$0.40^{b,c}$	0.23^{a}	$0.39^{b,c}$	0.47^{c}	0.04	0.002
P	1.1^{a}	1.2^{a}	1.1^{a}	1.1^{a}	1.6^{a}	3.6^{b}	6.5^{c}	7.3^{d}	0.2	<0.001

In each row, means with different superscript letter differ significantly.

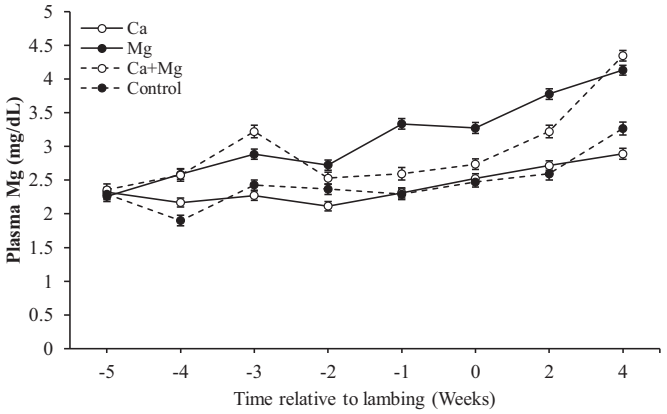

Fig. 1. Highest concentration of plasma magnesium (Mg) in ewes fed with magnesium supplement from 5 weeks prior to lambing (−5 W) to 4 weeks post lambing (+4 W). Error bars represent standard errors of means.

to the pre-lambing time points ($P < 0.001$), while the urinary Mg: creatinine ratio was lowest at +12 h ($P < 0.001$) (Table 2). Treatment did not have a significant effect on urine Ca: Creatinine ratio. Magnesium excretion through urine was greater in the Mg supplemented groups (Mg (0.43 ± 0.042) and Ca + Mg (0.53 ± 0.042)) compared to other groups (Ca (0.27 ± 0.041) and control (0.32 ± 0.045)) ($P < 0.001$). Phosphorous excretion through urine was significantly greater in the control group (12.0 ± 1.24) ($P = 0.001$) compared to supplemented groups (5.2 ± 1.23 for Ca group, 8.9 ± 1.23 for Mg group, 8.8 ± 1.22 for Ca + Mg group,). The P excretion in the Ca + Mg group tended to be greater than the Ca group ($P = 0.067$).

Parathyroid hormone, 1,25 dihydroxyvitamin D_3, 25 hydroxy vitamin D_3 concentration in ewes

The plasma concentration of PTH, $1,25(OH)_2D_3$ and $25(OH)D_3$ in different treatment groups are shown in Table 3. The plasma concentration of PTH, $1,25(OH)_2D_3$ and $25(OH)D_3$ was lowest at +2 W ($P \leq 0.002$). Ewes from the Ca + Mg group had the lowest mean concentration of $1,25(OH)_2D_3$ than the other groups ($P = 0.005$) and the mean concentration of PTH in this group tended to be lower than the Mg group ($P = 0.06$) (Table 3). However, the time × treatment interaction for the concentration of PTH, $1,25(OH)_2D_3$ and $25(OH)D_3$ was not significant.

Immune regulation in ewes as a response to calcium and magnesium supplementation

Treatment had no effect on oxidative burst response and phagocytosis function of leukocytes in ewes. Oxidative burst response of

Table 3
Plasma mean (± SE) concentration of parathyroid hormone (PTH) (pg/mL), 1, 25 dihydroxy Vitamin D (pg/mL), and 25 Hydroxy Vitamin D (ng/mL) in ewes of different treatment groups at different time points.

	Control	Ca	Mg	Ca + Mg	SE	*P*-value
PTH	$55.1^{a,b}$	$78.9^{a,b}$	100.0^{c}	39.0^{a}	22.8	0.060
$25(OH)D_3$	120.8	105.2	154.9	70.5	29.6	n.s.
$1,25(OH)D_3$	195.5^{b}	182.0^{b}	241.5^{b}	118.5^{a}	31.5	0.005
	−5W	−1W	+12H	+2W		
PTH	75.9^{b}	84.9^{b}	79.8^{b}	55.0^{a}	8.2	0.002
$25(OH)D_3$	119.1^{b}	107.6^{b}	132.4^{b}	85.1^{a}	10.2	0.001
$1,25(OH)D_3$	208.9^{b}	207.1^{b}	170.6^{b}	93.1^{a}	33.9	0.001

In each row, means with different superscript letter differ significantly.

leukocytes in ewes improved from the beginning of lambing through to four weeks post lambing ($P < 0.001$; Table 4). The phagocytic function of leukocytes was highest at +12 h compared to the other time points ($P < 0.001$; Table 4).

Mean TAC (μmol HCl/ml) in ewes was lowest at +12 h and was the highest at +4 W ($P < 0.001$; Table 4). The mean ROM (Carr U) concentration and mean OSi (ROM/TAC) ($P < 0.001$) at −1 W and at +12 h were higher compared to the other time points (Table 4). There was no treatment and time × treatment interaction effect on these parameters at any time point.

Minerals profile in lamb plasma

Plasma Ca and P concentration in lambs increased from +12 h (10.1 ± 0.22 mg/dL) to +2 W (11.4 ± 0.26 mg/dL) but then decreased significantly to +4 W (9.7 ± 0.23 mg/dL) ($P < 0.001$). Treatment and the time × treatment interaction for both Ca and P were not significant. Plasma Mg in lambs decreased over time ($P < 0.001$). There was a significant 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 (Table 5). Plasma Mg concentration significantly decreased over time ($P < 0.001$).

Immunity status of lambs

There was a significant increase in oxidative burst response of leukcoytes in lambs over time ($P = 0.015$). The treatment × time interaction tend to be significant ($P = 0.051$), with the Ca group having a greater oxidative burst response at +4 W compared to the other groups at this time point (Fig. 2). Phagocytic function of leukocytes was the greatest at +4 W (10.1 ± 1.11) compared to +12 h (5.9 ± 1.13) and +2 W (6.5 ± 1.14) ($P = 0.00$). However, phagocytic function of

Table 4
Changes in oxidative burst (RLU luminescence), phagocytosis (%), total antioxidant capacity (TAC) (HCl/ml), reactive oxygen metabolites (ROM) (Carr U) and oxidative stress index (OSi) (ROM/TAC) in ewes from 5 weeks prior to lambing to 4 weeks post lambing (Mean ± SE).

	−5 W	−4 W	−3 W	−2 W	−1 W	+12 H	+2 W	+4 W	SE	*P*-value
Oxidative burst	2208[a]	3199[a,b]	3926[b]	5358[c]	2761[a]	9506[e]	7871[d]	11272[f]	500	$P < 0.001$
Phagocytosis	14.8[b]	12.6[a,b]	11.1[a]	10.5[a]	12.6[a,b]	19.4[c]	11.0[a]	11.4[a]	1.1	$P < 0.001$
TAC	140[a]	363[d]	324[c,d]	310[c]	328[c,d]	231[b]	299[c]	372[d]	20.5	$P < 0.001$
ROM	141[a,c]	153[b,c]	119[a]	135[a,c]	370[d]	387[d]	118[a]	128[a,b]	11.35	$P < 0.001$
OSi	1.31[b]	0.56[a]	0.39[a]	0.50[a]	1.13[b]	1.67[c]	0.60[a]	0.44[a]	0.11	$P < 0.001$

In each column, means with different superscript letter differ significantly.

leukocytes did not differ with treatment and the time × treatment interaction significantly.

Treatment did not have a significant effect on colostrum IgG concentration. The plasma concentration of IgG at +2 W (20.1 ± 1.48 μg/ml) in lambs was greater ($P < 0.001$) than +12 h (14.0 ± 1.74 μg/ml) and +4 W (11.7 ± 1.32 μg/ml). Plasma IgG concentration tended to be greater in the Ca group (19.4 ± 1.68 μg/ml) compared to the Mg group (14.7 ± 1.98 μg/ml), the Ca+Mg group (13.6 ± 1.69 μg/ml) and the control group (14.2 ± 2.38 μg/ml) ($P = 0.055$). The time × treatment interaction showed that plasma IgG concentration tended to be greater in lambs from Ca group at +4 W ($P = 0.097$).

The mean concentration of TAC in lambs significantly decreased from +12 h (442.4 ± 18.44 μmol Hcl/ml) to +2 W (325.0 ± 15.78 μmol Hcl/ml) ($P < 0.001$) but did not change significantly from +2 W to +4 W (293.6 ± 16.01 μmol Hcl/ml). Lambs in the Mg and Ca+Mg groups had greater mean TAC compared to the Ca and control groups ($P = 0.04$; Fig. 3). However, the interaction time × treatment was not significant. Mean concentration of ROM in lambs changed significantly ($P < 0.001$) over time. Plasma ROM concentration in lambs was the greatest at +2 W (262.8 ± 14.50 Carr U) than +4 W (147.3 ± 13.02 μmol Carr U) and +12 h (53.9 ± 13.58 Carr U). However, no significant effect of treatment was observed on mean concentration of ROM ($P < 0.001$). Oxidative stress index in lambs at +2 W was significantly greater than the other time points ($P < 0.001$). Lambs in Mg groups experienced lower OSi compared to other groups at + 2 W ($P = 0.031$; Fig. 4).

Effect of maternal supplementation with calcium and magnesium on live weight of lambs

Live weight of lambs increased significantly from +12 h (4.5 ± 1.04 kg) to +2 W (8.1 ± 1.03 kg) and +4 W

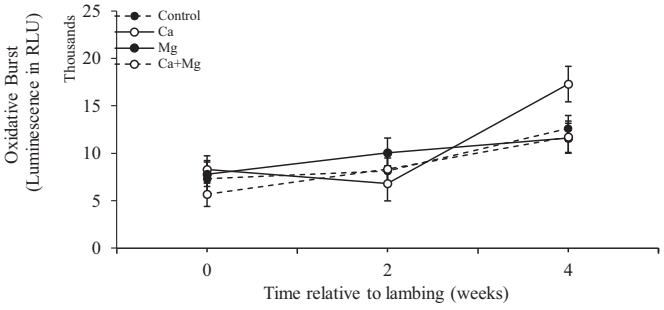

Fig. 2. Improvement of oxidative burst response of leukocytes in lambs from +12 h to +4 W as a result of maternal calcium supplementation. Error bars represents standard error of means.

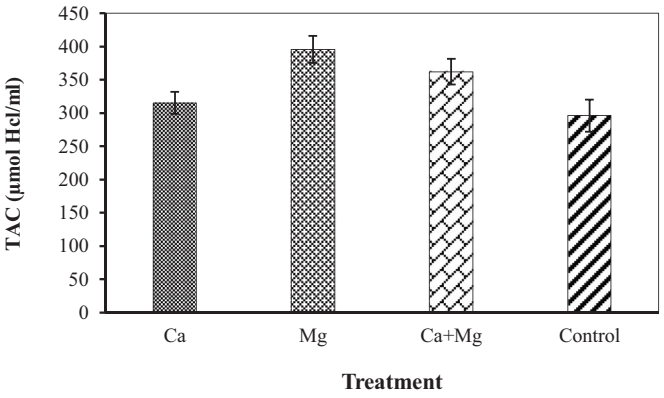

Fig. 3. An improvement in total antioxidant capacity in lambs born from mothers supplemented with magnesium in compare with calcium supplemented group and the control group. Error bars represents standard error of means.

Table 5
Plasma calcium (Ca), magnesium (Mg) and phosphorous (P) concentration in lambs at 12 h after birth (+12 h), 2 weeks of age (+2 W) and 4 weeks of age (+ 4 W) as a result of maternal supplementation with calcium and magnesium in late pregnancy and early lactation (Mean ± SE).

	Time			*P*-value		
	+12 H	+2 W	+4 W	Time	Treatment	Time × treatment
Plasma Ca						
Control	11.1 ± 0.4	11.7 ± 0.4	10.5 ± 0.4	<0.001	n.s.	n.s.
Ca	10.7 ± 0.4	10.9 ± 0.4	10.1 ± 0.4			
Mg	10.6 ± 0.4	11.6 ± 0.4	10.3 ± 0.4			
Ca+Mg	10.8 ± 0.4	11.6 ± 0.7	8.1 ± 0.6			
Plasma Mg						
Control	2.7 ± 0.1	2.6 ± 0.2	1.1 ± 0.2[a]	<0.001	0.023	0.001
Ca	2.6 ± 0.1	2.4 ± 0.1	2.1 ± 0.1[b]			
Mg	2.9 ± 0.1	2.3 ± 0.1	2.2 ± 0.1[b]			
Ca+Mg	2.6 ± 0.1	2.4 ± 0.1	2.1 ± 0.1[b]			
Plasma P						
Control	14.9 ± 0.9	21.2 ± 1.7	15.3 ± 1.3	<0.001	0.037	n.s.
Ca	14.7 ± 0.8	21.6 ± 1.0	19.1 ± 1.0			
Mg	12.9 ± 0.8	20 ± 0.9	19.5 ± 0.9			
Ca+Mg	15 ± 1.1	21.8 ± 1.0	20.8 ± 1.0			

In each column, means with different superscript letter differ significantly.

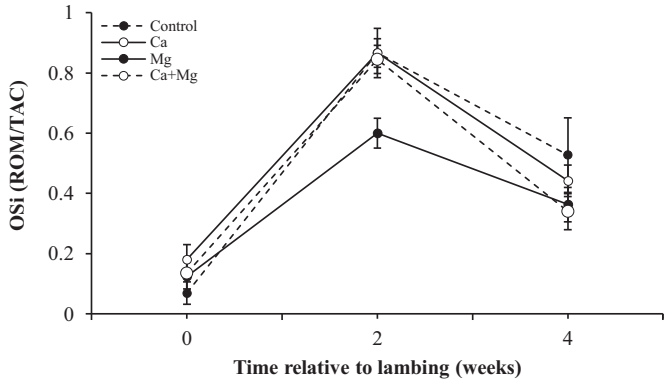

Fig. 4. Changes in oxidative stress in lambs from lambing to 4 weeks of age as a result of supplementation with calcium and magnesium. Error bars represents standard error of means.

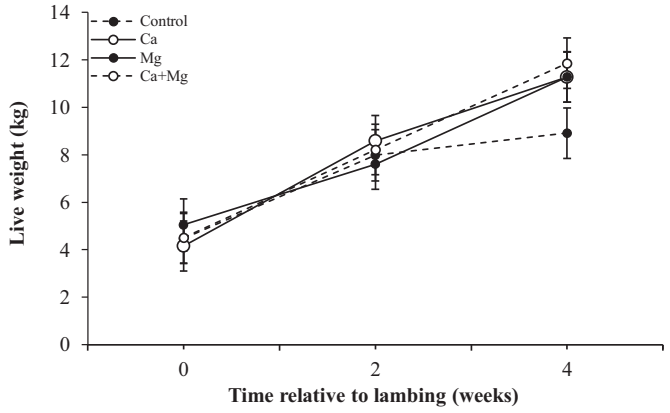

Fig. 5. Effect of maternal calcium and magnesium supplementation on live weight gain (kg) in lambs from lambing to 4 weeks of age. Error bars represents standard errors.

(10.5 ± 1.03 kg) ($P < 0.001$). Also, the live weight of single raised lambs (8.5 ± 1.03 kg) was greater than the twin raised lambs (7.0 ± 1.02 kg; $P = 0.007$). There was a significant treatment × time interaction for lamb weight ($P = 0.008$) as lambs in the supplemented groups had greater weight gain compared to the lambs from the control group ($P = 0.007$), with most of this benefit being apparent in the period from 2 to 4 weeks of age (Fig. 5). The average daily weight gain of treatment groups (from birth to 4 weeks of age) was 204 g/lamb/d (24%), 207 g/lamb/d (21%), 245 g/lamb/d (24%) for Ca, Mg and Ca + Mg groups, respectively, which was greater than 148 g/lamb/d (15%) for the control group ($P < 0.001$).

Discussion

Results of this study demonstrated that mineral supplementation provided to pregnant and lactating ewes improved the immune status and the live weight gain of lambs from birth to 4 weeks of age, supporting our hypothesis that provision of maternal mineral supplements above the requirements improve the health status and production of lambs. The concentration of Ca and Mg in the customised pellet in this study was above animal requirements. According to author's knowledge, this study is the first study that has evaluated the effect of mineral supplementation on health of ewes and lambs under a control feeding system. Thus, further study is required to optimize desire concentration of Ca and Mg in the feed on offer to maximize the profit

Supplementation of ewes with Mg improved plasma Mg concentration in ewes. Plasma Mg concentration is regulated by ruminal absorption and kidney excretion and is not under tight hormonal control. The rumen is the main site of Mg absorption (Greene, Fontenot & Webb, 1983) and active transport is the primary mechanism of Mg absorption from rumen under high luminal Mg concentration (Ram, Schonewille, Martens, Van't Klooster & Beynen, 1998). Therefore, a high concentration of Mg in the rumen is an impelling cause for Mg absorption (De Baaij, Hoenderop & Bindels, 2012).

Lower plasma concentration of hormones (PTH, $1,25(OH)_2D_3$ and $25OHD_3$) and high concentration of Ca in plasma at +2 W suggests that ewes had adequate Ca available at this time point to meet requirements which were in line with findings of Kadzere, Llewelyn and Chivandi (1997). However, low Ca concentration at pre-lambing acts as a signal to switch on the hormone mediated mechanisms so as to meet high Ca demands of ewes during late gestation which was evident with high plasma concentration of PTH, $1,25(OH)_2D_3$ and $25OHD_3$ (Schlumbohm & Harmeyer, 2003).

The low concentration of hormones like PTH $1,25(OH)_2D_3$ in the Ca + Mg group suggests that supplying Ca and Mg to ewes in the Ca + Mg group improved their ability to maintain Ca homeostasis, likely through increased availability of Ca in the diet facilitating intestinal Ca absorption and decreasing the demand for Ca regulating mechanisms (Goff, 2018). When dietary Ca is sufficient for the high Ca demands of ewes at late gestation, passive absorption of Ca from intestine will provide pregnant ewes with sufficient amount of Ca to meet body requirements (Schroder et al., 2015). Thus, the regulatory mechanisms of Ca such as PTH, $1,25(OH)_2D_3$ and $25OHD_3$ are deactivated, because body does not need extra Ca to be reabsorbed from kidney or mobilised from bone to meet requirements.

Ewes in our study underwent a period of immune suppression which was evidenced by high oxidative stress at lambing (Abuelo, Hernández, Benedito & Castillo, 2013). Oxidative stress was induced in ewes at the time of lambing due to an imbalance between the concentration of oxidants and antioxidants (Abuelo, Hernández, Benedito & Castillo, 2015) which happened at lambing time in this study. Oxidative stress during late gestation and at lambing is a common phenomenon due to the high hormonal and metabolic fluctuations, and also onset of inflammatory processes caused by parturition (Anugu et al., 2013). These findings are in line with the findings of Nawito, Hameed, Sosa and Mahmoud (2016).

The results of study showed that the highest plasma concentration of TAC in lambs was at 12 h after birth and it significantly decreased to 2 weeks of age suggesting that the high mean TAC at birth was a result of passive immunity transferred by the colostrum.

Maternal supplementation from one month prior to lambing to one month post lambing significantly boosted the immune response in lambs. Improvement of mean TAC in lambs from maternal supplementation with Mg in our study (both the Mg and Ca + Mg treatments) could be associated with passive transfer of TAC through the consumption of colostrum. 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 concentration of TAC in colostrum and evaluate the effect of Mg supplementation on TAC concentration in colostrum. 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 week of age which is in agreement with the study of Abuelo, Perez-Santos, Hernandez and Castillo (2014), in which calves showed greater mean ROM at weeks after birth. It seems that the high concentration of TAC presented at birth were capable of neutralizing the ROM generated at the same time in lambs in our study, because antioxidants are the main elements that neutralize ROM (Abuelo et al., 2015; Albera & Kankofer, 2011).

Leukocyte function for both oxidative burst response and phagocytosis in lambs increased over time. We postulate that this outcome was most likely due to normalization of leukocyte function to the adult

level with age. In human neonates, there is a lack in the function of neutrophil cells after birth due to insufficient number of membrane surface receptors, inefficient signal transduction and delayed mobilization of intracellular Ca (Makoni, Eckert, Anne Pereira, Nizet & Lawrence, 2016). 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. Mobilised Ca results in an efficient signal transduction which sped up the process of leukocyte maturation. Cytosolic Ca^{2+} is an ubiquitous signal, pivotal in many signal transduction pathways and controlling a wide range of cellular activities such as proliferation and differentiation (Melendez, 2005). These findings were further supported with greater IgG concentration in lambs from Ca supplementation at 4 weeks of age compared to the other groups at this time point. This could be related to the effect of Ca in leukocyte maturation and accordingly greater IgG synthesis by B-cells. Future research should evaluate Ca concentration in mature and immature leukocytes to investigate the mechanisms involved in leukocyte maturation.

An improvement in immune system function of lambs evidenced by antioxidant capacity and oxidative burst response of leukocytes, appears to be one of the contributing factors in the lamb's weight gain of the supplemented groups. High metabolic rate of growing tissue in growing lambs produces elevated levels of free radicals which should be counteracted by antioxidants (Chauhan et al., 2016). Besides, growing lambs exposed to more pathogens as they grow which require an efficient leukocyte function to be able to fight against the pathogens (Anugu et al., 2013; Dang et al., 2013). In addition, improved immunity status of lambs has been shown to increase the milk consumption of lambs and the nutrient absorption from the intestine (Walkden-Brown & Kahn, 2002) and consequently can affect lamb weight gain. Weight of lambs at around one month of age is an important factor that governs weaning weight and weaning time. There is no fixed age for weaning lambs and the size and weight of lamb at weaning is generally more important than its actual age. Lighter weaning weight of lambs makes mating management of hoggets difficult (Kenyon, Morel & Morris, 2004). Moreover, early weaning gives ewes a greater period to rest and recover their body condition (Arnold, Wallace & Maller, 1979; Lee, Majluf & Gordon, 1991; Silva et al., 2014) which may be a good strategy to increase reproductive performance of ewes.

Conclusion

Supplementation of ewes with Ca and Mg above published requirements improves the Ca homeostatic mechanism and plasma Mg concentration in ewes. Maternal supplementation with Ca and Mg had beneficial impacts on lamb production by improving immune response and increasing lamb live weight of lambs at 4 weeks of age.

Care and use of animals

The study was conducted at Charles Sturt University, Wagga Wagga, NSW, Australia from April 2017 to August 2017. The study was approved by the Charles Sturt University Animal Care and Ethics Committee (protocol number A16077).

Declaration of Competing Interest

There is no conflict of interest.

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References

Abuelo, A., Hernández, J., Benedito, J. L., & Castillo, C. (2013). Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. *Animal: An International Journal of Animal Bioscience, 7*, 1374–1378. https://doi.org/10.1017/S1751731113000396.

Abuelo, A., Hernández, J., Benedito, J. L., & 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. https://doi.org/10.1111/jpn.12273.

Abuelo, A., Perez-Santos, M., Hernandez, J., & Castillo, C. (2014). Effect of colostrum redox balance on the oxidative status of calves during the first 3 months of life and the relationship with passive immune acquisition. *Veterinary Journal, 199*, 295–299. https://doi.org/10.1016/j.tvjl.2013.10.032.

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. https://doi.org/10.1111/j.1439-0531.2010.01737.x.

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.

Anugu, S., Petersson-Wolfe, S. C., Combs, G. F., & Petersson, K. H. (2013). Effect of vitamin e on the immune system of ewes during late pregnancy and lactation. *Small Ruminant Research, 111*, 83–89. https://doi.org/10.1016/j.smallrumres.2012.10.010.

Arnold, G. W., Wallace, S. R., & Maller, R. A. (1979). Some factors envolved in natural weaning processes in sheep. *Applied Animal Ethology, 5*, 43–50. https://doi.org/10.1016/0304-3762(79)90006-3.

Bernabucci, U., Ronchi, B., Lacetera, N., & Nardone, A. (2005). Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *Journal of Dairy Science, 88*, 2017–2026. https://doi.org/10.3168/jds.S0022-0302(05)72878-2.

Bicalho, M. L., Lima, F. S., Ganda, E. K., Foditsch, C., Meira, E. B. S., Jr, & Machado, V. S. (2014). Effect of trace mineral supplementation on selected minerals, energy metabolites, oxidative stress, and immune parameters and its association with uterine diseases in dairy cattle. *Journal of Dairy Science, 97*, 4281–4295. https://doi.org/10.3168/jds.2013-7832.

Boland, T. M., Brophy, P. O., Callan, J. J., Quinn, P. J., Nowakowski, P., & Crosby, T. F. (2016). The effects of mineral-block components when offered to ewes in late pregnancy on colostrum yield and immunoglobulin g absorption in their lambs. *Animal Science, 79*, 293–302. https://doi.org/10.1017/S1357729800090159.

Brozos, C., Mavrogianni, V. S., & Fthenakis, G. C. (2011). Treatment and control of periparturient metabolic diseases: Pregnancy toxemia, hypocalcemia, hypomagnesemia. *Veterinary Clinics: Food Animal Practice, 27*, 105–113. https://doi.org/10.1016/j.cvfa.2010.10.004.

Campion, F. P., McGovern, F. M., Lott, S., Fahey, A. G., Creighton, P., & Boland, T. M. (2016). Comparison of energy rationing systems for late gestation ewes: Impacts on ewe and lamb performance1. *Journal of Animal Science, 94*, 3441–3456. https://doi.org/10.2527/jas.2016-0522.

Chauhan, S. S., Ponnampalam, E. N., Celi, P., Hopkins, D. L., Leury, B. J., & Dunshea, F. R. (2016). High dietary vitamin e and selenium improves feed intake and weight gain of finisher lambs and maintains redox homeostasis under hot conditions. *Small Ruminant Research, 137*, 17–23. https://doi.org/10.1016/j.smallrumres.2016.02.011.

Dang, A. K., Prasad, S., De, K., Pal, S., Mukherjee, J., Sandeep, I. V. R., et al. (2013). Effect of supplementation of vitamin E, copper and zinc on the in vitro phagocytic activity and lymphocyte proliferation index of peripartum sahiwal (Bos indicus) cows. *Journal of Animal Physiology and Animal Nutrition, 97*, 315–321. https://doi.org/10.1111/j.1439-0396.2011.01272.x.

De Baaij, J. H. F., Hoenderop, J. G. J., & Bindels, R. J. M. (2012). Regulation of magnesium balance: Lessons learned from human genetic disease. *Clinical Kidney Journal, 5*, i15–i24. https://doi.org/10.1093/ndtplus/sfr164.

Ducusin, R. J. T., Uzuka, Y., Satoh, E., Otani, M., Nishimura, M., Tanabe, S., et al. (2003). Effects of extracellular Ca2+ on phagocytosis and intracellular Ca2+ concentrations in polymorphonuclear leukocytes of postpartum dairy cows. *Research in Veterinary Science, 75*, 27–32. https://doi.org/10.1016/S0034-5288(03)00038-9.

Feske, S. (2007). Calcium signalling in lymphocyte activation and disease. *Nature Reviews Immunology, 7*, 690–702. https://doi.org/10.1038/nri2152.

Freer, M., Dove, H., & Nolan, J. E. (2007). *Nutrient requirements of domesticated ruminants.* Melbourne: CSIRO publishing.

Goff, J. P. (2018). Invited review: Mineral absorption mechanisms, mineral interactions that affect acid-base and antioxidant status, and diet considerations to improve mineral status. *Journal of Dairy Science, 101*, 2763–2813. https://doi.org/10.3168/jds.2017-13112.

Greene, L., Fontenot, J., & Webb, K. (1983). Site of magnesium and other macromineral absorption in steers fed high levels of potassium. *Journal of Animal Science, 57*, 503–510. https://doi.org/10.2527/jas1983.572503x.

Hernández-Castellano, L. E., Argüello, A., Almeida, A. M., Castro, N., & Bendixen, E. (2015). Colostrum protein uptake in neonatal lambs examined by descriptive and

quantitative liquid chromatography-tandem mass spectrometry. *Journal of Dairy Science, 98*, 135–147. https://doi.org/10.3168/jds.2014-8143.

Hoogendoorn, C., Betteridge, K., Costall, D., & Ledgard, S. (2010). Nitrogen concentration in the urine of cattle, sheep and deer grazing a common ryegrass/cocksfoot/white clover pasture. *New Zealand Journal of Agricultural Research, 53*, 235–243. https://doi.org/10.1080/00288233.2010.499899.

Jefferies, B. C. (1961). Body condition scoring and its use in management. *Tasmanian Journal of Agriculture, 32*, 19–21. https://doi.org/10.1080/00288233.1998.9513337.

Kadzere, C. T., Llewelyn, C. A., & Chivandi, E. (1997). Plasma progesterone, calcium, magnesium and zinc concentrations from oestrus synchronization to weaning in indigenous goats in zimbabwe. *Small Ruminant Research, 24*, 21–26. https://doi.org/10.1016/S0921-4488(96)00933-9.

Kenyon, P. R., Blair, H. T., Jenkinson, C. M. C., Morris, S. T., Mackenzie, D. D. S., & Peterson, S. W. (2009). The effect of ewe size and nutritional regimen beginning in early pregnancy on ewe and lamb performance to weaning. *New Zealand Journal of Agricultural Research, 52*, 203–212. https://doi.org/10.1080/00288230909510505.

Kenyon, P. R., Morel, P. C. H., & Morris, S. T. (2004). Effect of liveweight and condition score of ewes at mating, and shearing mid-pregnancy, on birthweights and growth rates of twin lambs to weaning. *New Zealand Veterinary Journal, 52*, 145–149. https://doi.org/10.1080/00480169.2004.36419.

Laires, M. J., & Monteiro, C. (2008). Exercise, magnesium and immune function. *Magnesium Research, 21*, 92–96. https://doi.org/10.1684/mrh.2008.0136.

Lee, P. C., Majluf, P., & Gordon, I. J. (1991). Growth, weaning and maternal investment from a comparative perspective. *Journal of Zoology, 225*, 99–114. https://doi.org/10.1111/j.1469-7998.1991.tb03804.x.

Makoni, M., Eckert, J., Anne Pereira, H., Nizet, V., & Lawrence, S. M. (2016). Alterations in neonatal neutrophil function attributable to increased immature forms. *Early Human Development, 103*, 1–7. https://doi.org/10.1016/j.earlhumdev.2016.05.016.

Martinez, N., Risco, C. A., Lima, F. S., Bisinotto, R. S., Greco, L. F., & Ribeiro, E. S. (2012). Evaluation of peripartal 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. https://doi.org/10.3168/jds.2012-5812.

Masters, D. G., Hancock, S., Refshauge, G., Robertson, S. M., McGrath, S., Bhanugopan, M., et al. (2018). Mineral supplements improve the calcium status of pregnant ewes grazing vegetative cereals. *Animal Production Science, 59*, 1299–1309. https://doi.org/10.1071/AN17403.

McClure, S. (2004). Mineral nutrition and its effects on gastrointestinal immune function of sheep. *Animal Production Science, 43*, 1455–1461. https://doi.org/10.1071/EA03002.

Melendez, A. J. (2005). *Calcium signaling during phagocytosis. Molecular mechanisms of phagocytosis*. Boston, MA: Springer US117–132.

Nawito, M. F., Hameed, A. R. A. E., Sosa, A. S. A., & Mahmoud, K. G. M. (2016). Impact of pregnancy and nutrition on oxidant/antioxidant balance in sheep and goats reared in south sinai. *Veterinary World, 9*, 801–805. https://doi.org/10.14202/vetworld.2016.801-805.

Nonnecke, B. J., Kimura, K., Goff, J. P., & Kehrli, M. E., Jr. (2003). Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. *Journal of Dairy Science, 86*, 2359–2368. https://doi.org/10.3168/jds.S0022-0302(03)73829-6.

Ram, L., Schonewille, J. T., Martens, H., Van't Klooster, A. T., & Beynen, A. C. (1998). Magnesium absorption by wethers fed potassium bicarbonate in combination with different dietary magnesium concentrations. *Journal of Dairy Science, 81*, 2485–2492. https://doi.org/10.3168/jds.S0022-0302(98)70140-7.

Schlumbohm, C., & Harmeyer, J. (2003). Hypocalcemia reduces endogenous glucose production in hyperketonemic sheep. *Journal of Dairy Science, 86*, 1953–1962. https://doi.org/10.3168/jds.S0022-0302(03)73783-7.

Schroder, B., Wilkens, M.R., .Ricken, G.E., .Leonhard-Marek, S., Fraser, D.R., .& Breves, G. (2015). Calcium transport in bovine rumen epithelium as affected by luminal ca concentrations and ca sources. 3, e12615–e12615. 10.14814/phy2.12615.

Silva, C. J. A. D., Fernandes, S. R., Silva, M. G. B. D., Monteiro, A. L. G., Poli, C. H. E. C., Prado, O. R., et al. (2014). Early weaning and concentrate supplementation strategies for lamb production on tifton-85 pasture. *Revista Brasileira de Zootecnia, 43*, 428–435. https://doi.org/10.1590/S1516-35982014000800005.

Thirunavukkarasu, M., Kathiravan, G., Kalaikannan, A., & Jebarani, W. (2010). Quantifying economic losses due to milk fever in dairy farms. *Agricultural Economics Research Review, 23*, 77–81.

Tygesen, M. P., Nielsen, M. O., Nørgaard, P., Ranvig, H., Harrison, A. P., & Tauson, A.-. H. (2008). Late gestational nutrient restriction: Effects on ewes' metabolic and homeorhetic adaptation, consequences for lamb birth weight and lactation performance. *Archives of Animal Nutrition, 62*, 44–59. https://doi.org/10.1080/17450390701780276.

Walkden-Brown, S. W., & Kahn, L. P. (2002). Nutritional modulation of resistance and resilience to gastrointestinal nematode infection - a review. *Asian-Australasian Journal of Animal Sciences, 15*, 912–924. https://doi.org/10.5713/ajas.2002.912.

Wills, M. R. (1969). The urinary calcium/creatinine ratio as a measure of urinary calcium excretion. *Journal of Clinical Pathology, 22*, 287–290. https://doi.org/10.1136/jcp.22.3.287.