

## AWET Undergraduate Project Scholarship 2014 Summary Report

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Supplementation of Merino ewes with cholecalciferol in late pregnancy improves the Vitamin D status of ewes and lambs at birth but is not correlated with an improvement in immune function in lambs

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### Introduction

On average, 30% of all lambs born will die prior to weaning, and approximately 80% of lamb deaths occur in the first 48 to 72 hours of life (Miller *et al.* 2010; Oldham *et al.* 2011; Hawken *et al.* 2012; Hinch and Brien 2014; Paganoni *et al.* 2014). Lamb birthweight is the greatest contributor to lamb survival however, even when birthweights are optimised, lamb survival to weaning rarely exceeds 90% for singles, 75% for multiples and 60% for triplets (Oldham *et al.* 2011; Hinch and Brien 2014; Paganoni *et al.* 2014), suggesting that factors independent of birthweight must also influence lamb survival. Immune competency has been linked to survival in other neonatal mammals (Zhao *et al.* 2008; Merlot *et al.* 2013), however its role in the mortality of lambs is poorly understood.

Functional deficiencies in the innate and adaptive immune systems, including in phagocytosis and the development of antibody-mediated immunity, are known to predispose neonates to infection and the associated inflammation, which may cause tissue damage and/or dysfunction, and death, particularly in the perinatal period (Firth *et al.* 2005; Futata *et al.* 2012). Furthermore, an increased genetic potential for CFW has been reported to compromise lamb survival and may be due to competition between the immune system and wool follicles for essential nutrients (Lee *et al.* 1993; Liu *et al.* 2005; Williams 2011; Brien *et al.* 2014). Therefore, lambs with a higher genetic potential for fleece weight may have more compromised immune systems and be at increased risk of dying.

Vitamin D has been recognised to have several roles in the regulation of immune function and can enhance innate antimicrobial immune responses, whilst dampening excessive adaptive immune responses and inflammation (Lang *et al.* 2013). Maternal requirements for Vitamin D are increased during pregnancy (Lucas *et al.* 2008) and Vitamin D is transferred across the placenta to the fetus (Kovacs 2008; Lapillonne 2010). Increased Vitamin D levels in ewes and neonatal lambs may therefore be associated with a greater ability to control infection and/or limit infection-driven inflammation, and thus may contribute to improved lamb survival. Supplementation of ewes with Vitamin D during pregnancy may therefore increase the levels of Vitamin D in the ewe and her lamb/s, thereby improving innate and/or adaptive immunity in the ewe, and innate and/or passive immunity in the lamb.

This study therefore tested the hypotheses that; (i) Maternal supplementation of Merino ewes with cholecalciferol during late pregnancy increases the levels of Vitamin D in the ewe and lamb at birth, (ii) Maternal supplementation of Merino ewes with cholecalciferol during late pregnancy is correlated with increased innate phagocytic and adaptive antibody responses in the lamb, and (iii) The effect of maternal cholecalciferol supplementation on lamb immune function will be greater in lambs with a higher genetic potential for fleece weight.

### **Experimental design**

Two hundred Merino ewes aged between 4 and 7 years old were sourced from the 'Maternal Efficiency Flock'. The ewes were artificially inseminated with semen from four sires specifically selected to have different ASBVs for clean fleece weight at yearling age (YCFW) in order to assess the differences in immune responses between lambs with higher and lower genetic potential for fleece weight, where L1 and L2 are the sires with a low genetic potential for YCFW and H1 and H2 are the sires with a high genetic potential for YCFW. On day 111 pregnancy the ewes were allocated into three replicates of two treatment groups; Control or Vitamin D supplementation during late pregnancy. The Control group ( $n = 58$ ) consisted of ewes receiving no additional nutritional treatments. Ewes supplemented with Vitamin D ( $n = 53$ ) received an injection of cholecalciferol (Vitamin D<sub>3</sub>) at days 113 and 141 of pregnancy. On each occasion,  $1.0 \times 10^6$  IU cholecalciferol, in oil, was injected intramuscularly into the hind-limb.

Blood samples were collected from Vitamin D and Control ewes prior to treatments, on day 111 of pregnancy, for subsequent analysis of Vitamin D concentrations. At lambing ewes were intensively monitored in order for ewe and lamb sampling to occur prior to the lamb suckling following birth. At birth, all lambs were weighed, and their dam, sex and birth type was recorded ( $n = 154$ ). Blood samples were collected from all single-born lambs and the first lamb born only for those born in litters for subsequent Vitamin D and immunological analysis. Rectal temperatures and blood glucose concentrations of the lambs were measured where possible. Colostrum and blood samples were collected from all ewes for immunological and Vitamin D analysis respectively. Lambs were bled again at 1-, 4-, 6- and 14- weeks of age for subsequent Vitamin D and/or immunological analysis. Lambs were marked and given their primary vaccinations at 4-weeks of age and were weaned at 14-weeks of age. Ewes were weighed and body condition scored every 1-2 weeks prior to lambing and both ewes and lambs were weighed every 2-3 weeks between lambing and weaning.

The Vitamin D status' of the ewes and lambs was assessed by measuring the total concentration of 25-hydroxyvitamin D in plasma. Lamb immune function was assessed by analysing the functional capacity of phagocytes (monocytes and polymorphonuclear leukocytes), and the plasma immunoglobulin-G and anti-tetanus-toxoid antibody concentrations between birth and weaning.

Statistical analyses were performed using GENSTAT (VSN International 2012). The data was assessed using Restricted Maximum Likelihood and Generalized Linear Mixed Models, where

appropriate. Data was angular or log-transformed where necessary and is presented in the back-transformed state. Statistical significance was accepted at  $P < 0.05$ .

### Summary of Results

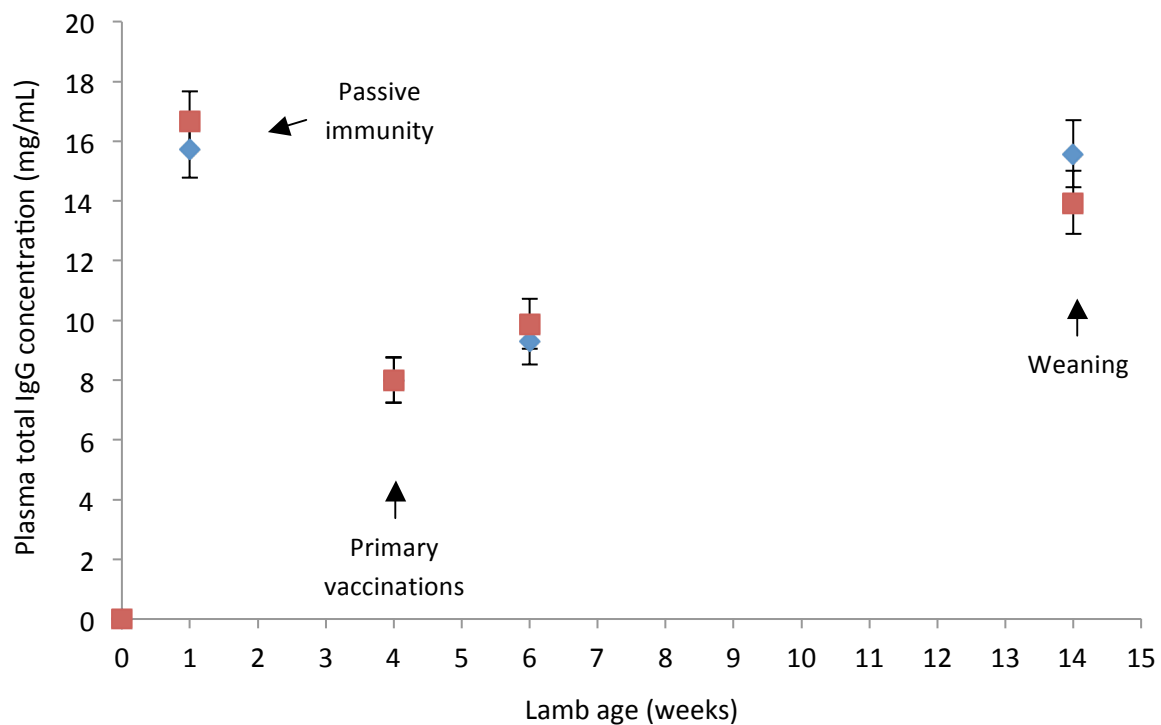
Maternal supplementation with cholecalciferol in late pregnancy increased the plasma concentrations of 25(OH)D in supplemented ewes by 74% at lambing and this doubled the plasma 25(OH)D concentrations in their lambs at birth (Table 1). The plasma 25(OH)D concentrations of ewes and lambs were positively correlated at lambing, confirming that 25(OH)D crosses the placenta in ewes to increase the plasma 25(OH)D concentrations of the fetus and neonate. These findings support our first hypothesis. However, lambs born to both Vitamin D-supplemented and Control ewes were likely to be Vitamin D-deficient at both birth and 4-weeks of age. There is no data available on critical plasma 25(OH)D concentrations in adult or neonatal sheep, but in humans plasma 25(OH)D concentrations of  $< 50$  nmol/L are generally classified as Vitamin D-deficient (Lucas *et al.* 2008; Holick 2009; Principi *et al.* 2013; Thiele *et al.* 2013).

**Table 2. Mean plasma total 25-hydroxyvitamin D concentrations (nmol/L) of Control and Vitamin D-supplemented ewes prior to supplementation (day 111 pregnancy), at lambing and 4-weeks after lambing, and of their lambs at birth and 4-weeks of age. The ewes were supplemented with cholecalciferol on days 113 and 141 of pregnancy. Values are presented in the back-transformed state where appropriate.**

	Control	Vitamin D	P value
<i>Ewes</i>			
Pre-treatment	66.3	72.5	0.259
Lambing	78.6	137.0	$< 0.001$
4-weeks	73.0	157.0	$< 0.001$
<i>Lambs</i>			
Birth	24.2	49.0	$< 0.001$
4-weeks	21.9	25.6	0.168

There were no significant differences between lambs from Control and Vitamin D-supplemented ewes in the percentages of phagocytic monocytes or PMNL in whole blood at birth (84.2 vs 85% and 60.0 vs 60.2%, respectively) or 4-weeks of age (98.6 vs 98.2% and 91.2 vs 92.8%, respectively). The concentration of IgG in the colostrum of Control and Vitamin D ewes did not differ significantly (80.4 vs 82.3 mg/mL) and there was no significant effect of maternal supplementation on the concentrations of IgG in the plasma of lambs between birth and weaning (Figure 1). Maternal supplementation had

no effect on the vaccine-specific antibody response against tetanus-toxoid in lambs between birth and weaning. Therefore our second hypothesis was rejected. The genetic potential of the lamb for YCFW had no significant effect on the competency of its immune system, therefore our third hypothesis was also rejected. However, the lack of improvement in the immune function of the lamb may be explained by the poor Vitamin D status of the lambs. We did not examine the antimicrobial killing capacities of phagocytes or the inflammatory responses in the lamb and therefore further research could investigate the relationship between the plasma Vitamin D levels in the lambs and these immune measures.



**Fig. 2.** Mean plasma total IgG concentrations ( $\pm$  least significant intervals) of Control (blue) and Vitamin D (red) lambs at birth and 1-, 4-, 6- and 14-weeks of age. The ewes were supplemented with cholecalciferol on days 113 and 141 of pregnancy. Values are presented in the back-transformed state.

Maternal supplementation with cholecalciferol in late pregnancy did not have any significant effect on the liveweights or body condition scores of the ewes between artificial insemination and weaning, and there was no effect of maternal supplementation or the genetic potential of the lamb for YCFW on the liveweights of lambs between birth and weaning or the rectal temperatures or blood glucose concentrations of lambs at birth. There was no significant difference in the survival of Vitamin D or Control lambs to weaning (80.8 vs 69.9% ;  $P = 0.186$ ). On average, only 4.6% of lambs born alive died within the first 72 hours of life in the present study, which is considerably lower than the expected mortality of 20 – 30% (Oldham *et al.* 2011; Hawken *et al.* 2012; Hinch and Brien 2014). We therefore expect that the limited number of lamb deaths during this high risk period was due to the

high level of intervention associated with intensive sampling at lambing. Nevertheless, lambs sired by L1 (87.9%) tended to have lower survival to weaning than lambs sired by H2 (72.3% ; P = 0.079), which represent the sires with the highest and lowest genetic potential for YCFW, respectively. Whilst the differences are unlikely to be related to differences in immune function, these findings support those of previous studies which have identified negative correlations between lamb survival and the genetic potential for CFW (Adams *et al.* 2006; Safari *et al.* 2007; Brien *et al.* 2014) and suggest further research into the correlation between CFW and lamb survival is warranted.

Overall our novel findings showed that supplementation of ewes with two large intramuscular doses of  $1 \times 10^6$  IU cholecalciferol in late pregnancy is inadequate in boosting the immune competency of the lamb and may be due to the immune system not being responsive to Vitamin D-supplementation if the lamb is in a Vitamin D-deficient state. Further research is therefore required to determine the requirements for Vitamin D in adult and neonatal sheep and additionally what levels of Vitamin D are required to optimize immune function. Subsequent research could then determine what approach to Vitamin D-supplementation is most effective at safely boosting the immune competency of the lamb and if this is associated with an improvement in lamb survival.

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