Breed and diet effects on ewe colostrum quality, lamb birthweight and the transfer of passive immunity

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Glossary of Terms and Abbreviations

Abbreviation	Term
ALP	Alkaline phosphatase
CP	Crude protein
d	Day(s)
DM	Dry matter
ELISA	Enzyme-linked immunosorbent assay
FcRn	Neonatal Fc Receptor
FPT	Failure of passive transfer
g	Gram(s)
GCT	Glutaraldehyde coagulation test
GGT	Gamma glutamyltransferase
h	Hour(s)
ha	Hectare(s)
IEL	Endometrial intraepithelial lymphocytes
Ig	Immunoglobulin
kg	Kilogram(s)
L	Litre(s)
LDH	Lactate dehydrogenase
MALT	Mucosa-associated lymphoid tissue
ME	Metabolisable energy
MEC	Mammary epithelial cells
MLA	Meat Livestock Australia
MJ	Megajoule(s)
mg	Milligram(s)
mL	Millilitre(s)
nm	Nanometre(s)
NRC	National Research Council
OD	Optical density
plgR	Poly immunoglobulin receptor
RID	Radial immunodiffusion
SST	Sodium sulphite turbidity test
µL	Microlitre(s)
ZST	Zinc sulphate turbidity assay

Abstract

Ruminant neonates are born agammaglobulinaemic, as no antibodies from the dam are transferred to the foetus via the placenta. Therefore, they are entirely dependent on gaining passive immunity from their mother after birth through the ingestion of colostrum. The colostrum immunoglobulins, especially IgG, in conjunction with the ability of the neonatal gut to allow unrestricted passage of the large immunoglobulin molecules, provide the newborn with passive immunity.

Previously, a number of studies have been undertaken analysing the effects that diet and breed have on bovine colostrum quality and the transfer of passive immunity in calves. However, there has been limited research on these effects in sheep. Thus the major aim of this study was to examine breed and diet effects on ewe immune status and colostrum quality, and the birth weight and/or the transfer of passive immunity in their lambs. From this it would be possible to determine if relationships exist between (1) the immune status (serum IgG concentration) of the ewe in late gestation and the quality of the colostrum she subsequently produces; and (2) the quality of colostrum consumed by lambs and their subsequent immune status. Both male and female lambs and singles and twins were sampled in this study to see if sex or birth type influenced the birth weight and the immune status of lambs. In addition, the effect of lamb birth weight on the immune status of lambs, and if either lamb birth weight or their immune status affected their survival was investigated.

This study involved Merino and Dorper ewes grazing dual-purpose wheat and canola in late gestation. The experimental design was a 2 x 2 factorial treatment structure, with two treatments and three replicates. A total of 24 pregnant ewes, 12 Merino and 12 Dorper, were randomly selected from a mob of 142 pregnant ewes, involved in a larger MLA trial. The main focus of this study was determination of the concentration of the immunoglobulin G (IgG) in ewe serum, colostrum and lamb serum, 24 h post-partum. Blood samples were taken from the jugular vein of the ewes and lambs, and ewes were hand-milked to collect a minimum of 5 mL of colostrum, 24 h after birth. The IgG concentrations of the ewe and lamb samples were quantified using a commercially available ovine ELISA assay.

Measurement of IgG concentrations by ELISA revealed there were no significant ($P > 0.05$) breed differences between Merino and Dorper ewes for the concentration of IgG in their serum and colostrum. The diet, dual-purpose wheat or canola that the ewes grazed also did not significantly ($P > 0.05$) influence ewe immune status or colostrum quality, 24 h post-partum. The colostral IgG concentration was not associated ($P > 0.05$) with serum IgG concentrations in both ewes and lambs. This suggests that the immune status of ewes cannot be used to predict the quality of colostrum they will produce and therefore the immune status of their lambs. The serum IgG concentration of ewes

was significantly ($P < 0.05$) associated with their live weight and body condition score one week pre-partum, although their colostral IgG concentration was not ($P > 0.05$). The breed genotype of ewes and the diet they grazed during late gestation did not affect ($P > 0.05$) the concentration of IgG in their lamb's serum or their birthweight. When fitted to a linear model, the interaction term of colostral IgG concentration modified by lamb birthweight did significantly affect ($P < 0.05$) lamb serum IgG concentration. Birth type significantly ($P < 0.05$) affected the birthweight of lambs, and single lambs (5.61 ± 0.306 kg) were significantly heavier than that of twin lambs (4.86 ± 0.139 kg). There was a highly significant ($P < 0.01$) relationship between lamb serum IgG concentration 24 h post-partum and their survival pre-weaning.

The results suggested that grazing Merino and Dorper ewes on dual-purpose wheat and canola during late gestation does not affect their serum IgG or colostral IgG concentrations 24 h after lamb birth. Also, the immune status of lambs was not influenced by ewe breed, the diet ewes consumed, colostrum quality, birth type, sex or birthweight. However, the immune status of lambs can be influenced by the quality of colostrum they consumed modified by their birthweight. Also, depending on whether lambs are born as a singleton or twin, their birthweight will either be heavier or lighter, respectively. Finally, lamb serum IgG concentrations > 6.96 mg/mL are beneficial to lamb survival in the pre-weaning period. Further research is needed to confirm the effect of different diets of varying qualities and types and different sheep breeds have on the immune status of ewes and the quality of colostrum they produce and the immune status of lambs and their birthweight. More studies are also needed to identify the key factors that affect the transfer of passive immunity in lambs and why there is such variability in their immune status 24 h post-partum.

Chapter 1. Introduction

One of the many challenges of being a livestock producer is to produce healthy newborns that will survive and perform to their full genetic potential. The long term productivity of an animal is linked to its immune status and that the immune and nutritional status of pregnant dams can have consequences on the health and performance of their offspring.

Newborn ruminants require a sufficient amount of colostrum within 48 h post-partum to survive (Stelwagen *et al*. 2009). The colostrum contains important antibodies (Pattinson *et al*. 1995) which provides a defence mechanism for newborn ruminants until their own immune system is established (Ahmad *et al*. 2000; Yilmaz and Kasikci 2013). There is a restricted time period for the transition of immunoglobulins into the small intestines of newborns as the intestinal epithelium (Galan-Malo *et al*. 2014) cannot absorb antibodies after 48 h post-partum (Yilmaz and Kasikci 2013). The quantity of colostrum produced by ewes is one of the determinants of colostrum intake by newborn lambs (O'Doherty and Crosby 1997) and there are potential differences both between and within breeds in colostral IgG concentrations (Tabatabaei *et al*. 2013).

Unsuccessful transfer of passive immunity in young animals results in higher susceptibility to diseases, increased mortalities and off-spring that do not perform to their full potential (Hashemi *et al*. 2008). Ahmad *et al.* (2000) found lambs that died during the neonatal period had significantly lower concentrations of immunoglobulins than lambs that survived the neonatal period. Furthermore, higher mortality rates were observed in lambs born to ewes that had a lower IgG content in their colostrum (Tabatabaei *et al*. 2013).

The diet consumed by pregnant ewes in late gestation can influence colostrum production and therefore the amount of colostral IgG available and absorbed by their lambs (O'Doherty and Crosby 1997; Banchero *et al.* 2006). The maternal nutritional status of the ewe can influence the gut permeability to immunoglobulins of their newborn lambs (Hodgson *et al*. 1997). Newborn lambs have a greater absorption of immunoglobulins when the dam is healthier. Furthermore, nutritional supplementation of dams can enhance the erythropoietic response and therefore improve offspring survival (Ahmad *et al*. 2000).

The birthweight of newborns can have a significant effect on their subsequent immunoglobulin concentrations. Newborns that have a lower birth weight tend to be weaker and therefore unable to or have more trouble suckling adequate amounts of colostrum to provide

sufficient levels of antibodies in their blood for initial immune protection (Ahmad *et al*. 2000; Hashemi *et al.* 2008).

Determining the effects that diet and breed have on the immune status of ewes, the quality of colostrum they produce, and the subsequent immune status of lambs will enable producers to make improved management decisions to improve productivity.

1.1 Hypotheses

The research was designed to test the following hypotheses:

1. The diet (dual-purpose wheat versus canola forage) consumed by ewes (Merino and Dorper) during late gestation impacts on their immune status and the subsequent quality of colostrum they produce.
2. When fed the same diet, breed differences exist in the immune status of ewes and the subsequent quality of colostrum they produce.
3. The diet (dual-purpose wheat versus canola forage) consumed by ewes (Merino and Dorper) during late gestation impacts on the birth weight and immune status of their lambs.
4. When ewes are fed the same diet, breed differences exist in the birth weight and immune status of their lambs.
5. A direct relationship exists between the quality of colostrum ingested, birthweight, the immune status and subsequent growth rate of lambs.

Chapter 2. Literature Review

2.1 Scope of the review

The provision of an adequate amount of high quality (high IgG concentration) maternal colostrum is essential for the health and survival of neonatal lambs. Because lambs are born agammaglobulinaemic they depend entirely on the absorption of maternal immunoglobulins from colostrum after birth to establish a functional immune system. Many factors influence the success of this transfer of immunity and these are the focus of this review.

2.2 Colostrum

Ruminant neonates rely entirely on colostrum and milk from their dam for survival (Wereme *et al.* 2001; Stelwagen *et al.* 2009). Ruminant colostrum is a mixture of lacteal secretions and constituents from blood serum, which consist of immunoglobulins and other serum proteins (Foley and Otterby 1978). Newborns acquire passive immunity through ingesting immunoglobulins contained in colostrum (Stelwagen *et al.* 2009). There are five classes of immunoglobulins, IgG, IgM, IgA, IgD and IgE; with IgG, IgA, IgM (Ahmad *et al*. 2000; Gapper *et al.* 2007; Hashemi *et al*. 2008) and IgE being actively transported into ovine colostrum (Hine *et al.* 2010). IgG is the most predominant (Thompson *et al.* 2013) and abundant (Hashemi *et al*. 2008) immunoglobulin, accounting for approximately 80-90% of all immunoglobulins in colostrum (Ingvarsson 1995; Mech *et al.* 2011). The transfer of immunoglobulins into ovine colostrum is most selective for IgG_1, followed by IgA, IgE, IgM and finally IgG_2 (Hine *et al.* 2010).

All immunoglobulin molecules are based around the same structure, consisting of two heavy polypeptide chains and two light polypeptide chains (Gapper *et al.* 2007). Each of the light chains is bound to one of the heavy chains by disulfide bridges, while the heavy chains are similarly bound to one another resulting in a Y shape. At the tip of each arm there are two identical antigen binding sites. These are unique for each different antibody so that it can only interact with a specific antigen (Sherwood *et al*. 2005).

There are two subclasses of IgG, IgG_1 and IgG_2 (Gapper *et al.* 2007). IgG_1 is the primary immunoglobulin found in ruminant colostrum (Weaver *et al.* 2000; Mayer *et al.* 2002; Hine *et al.* 2010) and is derived mainly from maternal circulation. IgG_2 is derived from blood or can be synthesised in the cells of the mammary gland (Gapper *et al.* 2007). IgG is responsible for pathogen clearance (Mech *et al.* 2011) and protects the neonate against bacteria and viruses, neutralises bacterial toxins, triggers

the complement system and when bound to antigens enhances the effectiveness of phagocytic cells (Tortora *et al*. 2006).

Both IgM and IgA provide protection against systemic infection (Mech *et al.* 2011). IgM makes up approximately 5-10% of the immunoglobulins in colostrum (Ingvarsson 1995). It is a much larger molecule than IgG, preventing it from moving around as freely as IgG does and therefore it generally remains in the blood vessels. IgM is involved in aggregating antigens and enhancing ingestion of target cells and appears first in response to a primary infection (Tortora *et al*. 2006) IgM is typically involved in the humoral immune response (Dudek *et al*. 2014).

IgA makes up approximately 10-15% of the antibodies in colostrum (Ingvarsson 1995), although it is the most common antibody in the body. It is secreted by plasma cells located beneath the body surfaces (Kacskovics 2004) and is found in mucous membranes and body secretions such as mucous, saliva, tears and milk. It functions as a neutralising antibody (Murphy *et al*. 2014) and mainly functions to prevent respiratory disease (Tortora *et al.* 2006). The poly immunoglobulin receptor (plgR) is responsible for the transport of IgA across the ovine mammary epithelium (Hine *et al.* 2010). Dudek *et al*. (2014) observed an increase in IgA concentrations in Holstein-Fresian calves born to dams vaccinated during gestation and suggested the increase was due to passive colostrum transfer and stimulation of mucosal immunity within mucosa-associated lymphoid tissue (MALT) to prevent pathogen colonisation.

Ruminant colostrum also contains various cellular (leukocytes, cytokines, macrophages, lymphocytes and natural killer cells) (Sangild 2003; Stelwagen *et al*. 2009; Yilmaz and Kasikci 2013) and humoral factors (lactoferrin, lysozymes and complements) (Yilmaz and Kasikci 2013), along with insulin-like growth factor (Sangild 2003; Ocak *et al.* 2005; Nowak and Poindron 2006), insulin and growth hormone, thyroxine, triiodothyronine, and prolactin (Yilmaz and Kasikci 2013), that aid in the survival of newborns.

During the last few weeks of gestation, plasma cells synthesise IgA, IgM and IgG in the submucosa of the mammary gland epithelium (Yilmaz and Kasikci 2013). The majority of immunoglobulins enter colostrum via a selective (Kacskovics 2004) receptor-mediated intracellular route. Some immunoglobulins can also enter via serum through intercellular tight junctions (Stelwagen *et al*. 2009). The neonatal Fc receptor (FcRn), a specific IgG receptor in the acinar and ductal mammary epithelial cells (MEC), transports IgG from the serum into the lactating mammary gland and is regulated by endocrine changes throughout gestation (Mayer *et al.* 2002; Yilmaz *et al.* 2011; Tabatabaei *et al*. 2013). Hence, the FcRn receptor has an important role in IgG metabolism

(Brujeni *et al*. 2010) and in preventing the degradation of IgG in maternal circulation (Kacskovics 2004). The FcRn receptors determine the concentration of IgG in colostrum (Weaver *et al.* 2000) and have different affinities for the subclasses of IgG (Baintner 2007). At 24 and 10 d pre-partum, FcRn expression was observed to be greatest in the acinar and ductal MEC of ovine dams (Mayer *et al.* 2002) and then at lactation expression ceases (Weaver *et al.* 2000). Hine *et al.* (2010) found FcRn expression to be up-regulated in dry ewes and ewes in late gestation and down-regulated during colostrogenesis and lactation.

In addition to its role in developing immunity in the neonate, colostrum is nutritionally significant as a first meal (Hernandez-Castellano *et al.* 2014). Compared to normal milk, colostrum has increased fat, vitamins (A, B_{12}, D, E) and protein concentrations and a lower lactose concentration (Pattinson *et al*. 1995; Yilmaz and Kasikci 2013; Hernandez-Castellano *et al.* 2014). Furthermore, normal milk in ruminants contains 12% total solids whereas colostrum contains 22% total solids (Yilmaz and Kasikci 2013). In addition to providing immunity, colostrum is critically important to provide energy to the neonate for thermoregulation (Yilmaz and Kasikci 2013).

2.2.1 Factors affecting colostrum quality

Colostrogenesis, the transfer of immunoglobulins from the dam's circulation into mammary secretions, begins one or two weeks pre-partum (Baintner 2007) and is under hormonal control by oestradiol and progesterone (Castro *et al.* 2011). High plasma progesterone is negatively associated with colostrum yield, and low nutrition during gestation can result in higher plasma progesterone concentrations, hence affecting the survival of lambs due to inadequate colostrum production (Nowak and Poindron 2006) and subsequent transfer of passive immunity. The IgG specific receptor on alveolar epithelial cells, involved in transporting IgG from blood into colostrum, is inactivated when prolactin (lactogenic hormone) increases during lactogenesis, thereby down regulating the transfer of immunoglobulins (Weaver *et al.* 2000; Castro *et al.* 2011). Shortly after parturition the secretion of immunoglobulins in colostrum ceases (Baintner 2007).

In the ewe, serum IgG concentration peaks during late gestation and significantly decreases post-partum (Mayer *et al.* 2002; Hine *et al.* 2010). The highest IgG concentration in colostrum is at 1 h post-partum and then rapidly declines at 12 and 24 h post-partum (Hashemi *et al.* 2008). However, the relationship between ewe (serum) IgG concentrations and IgG concentrations in colostrum is unclear. Tabatabaei *et al*. (2013) found colostral IgG concentrations were not associated with ewe serum IgG concentration whereas Hashemi *et al*. (2008) found that the IgG concentrations in ewe serum are significantly correlated with IgG concentrations in colostrum.

The concentration of IgG in colostrum can be influenced by various factors including breed, lactation number, age, health status, nutrition, body condition score at parturition and genetics as well as environmental factors (Gilbert *et al.* 1988; Hart *et al.* 2009).

Variations in colostrum quality (concentration of IgG) due to breed differences have been reported in both cattle and sheep (Castro *et al.* 2011). Dairy cattle produce increased amounts of colostrum of a higher quality compared to beef breeds (Yilmaz and Kasikci 2013). Tabatabaei *et al.* (2013) found considerable variation in colostral IgG concentrations between Shaul and Lori Bakhtyari ewes and suggested the breed differences in the quality of colostrum produced may be due to polymorphism in the FcRn gene. In cattle, FcRn polymorphisms have been associated with passive transfer of IgG; however, there is limited research in different sheep breeds on how FcRn causes differences in IgG concentrations and profiles (Brujeni *et al*. 2010)

Age of the dam can influence the quantity and quality of colostrum. Both ewes and cows have been reported to produce more colostrum with a higher content of immunoglobulins than younger animals (Yilmaz and Kasikci 2013). Colostrum from heifers is of a poorer quality when compared to older cows (Bielmann *et al*. 2010). However, there appears to be an interaction between the age and breed on colostrum quality. Most studies report a tendency for the older animals to produce a higher quality colostrum; however, in a comparison between Holstein and Guernsey cows, Godden (2008) found that whilst there was an increase in mean colostral content in the first, second and third lactations of Holstein cows there was no difference (in mean colostral content) between lactations in Guernsey cows.

Both parity and breed can influence IgG concentration in colostrum (Csapo *et al.* 1994). Bielmann *et al*. (2010) observed that as parity increased in Holstein cows, the Ig concentration of colostrum also increased. Weaver *et al.* (2000) found that cows in their third lactation had significantly higher immunoglobulin concentrations (81.5 mg/mL) than cows in their first, second and fourth lactation (59.1 mg/mL, 62.6 mg/mL and 74.9 mg/mL, respectively).

The condition of the dam's udder can also influence the quality of colostrum produced. Mastitis in ewes has been found to reduce serum immunoglobulin concentration, leading to a reduction in the concentration of immunoglobulins in colostrum for neonate absorption (Chirstley *et al.* 2003).

Dry matter (DM) intake has variable effects on the IgG concentration of colostrum and blood sera of ewes and lambs. Swanson *et al*. (2008) reported that ewes fed 60% of NRC requirements had an average IgG concentration in colostrum of 127.7 mg/mL, whilst ewes fed at 140% of NRC

requirements had a colostral IgG concentration of 99.9 mg/mL. Both these values were significantly higher than the control group (82.1 mg/mL) fed at 100% of requirements. However, both under-nutrition and over-nutrition of ewes from mid gestation can cause reductions in colostrum composition and yield. Over-nourishing pregnant ewes has been found to reduce the total IgG concentration in colostrum compared to moderately fed ewes (Castro *et al.* 2011). In addition, under-nutrition of ewes can lower the concentrations of lactose, lipids and protein in colostrum (Hashemi *et al.* 2008).

The quantity of colostrum produced can be a determinant of colostrum intake by newborn lambs (O'Doherty and Crosby 1997). Colostrum yield tends to increase between the period of 1 to 10 h post-partum, and then a reduction is seen from 10 to 18 h onwards (Boland *et al.* 2005). The effects of nutrition of the dam on the quantity and quality of colostrum produced are variable. Nutrition during gestation can influence the quantity of colostrum produced; dams well fed in late gestation tend to produce more colostrum, whereas, underfeeding in late gestation can result in a reduced total yield of colostrum (Swanson *et al*. 2008) in the first 18 h after birth (Hashemi *et al*. 2008). Meyer *et al.* (2011) found that high levels of selenium supplementation of pregnant ewes throughout gestation resulted in an increased colostrum yield compared with ewes only supplemented with adequate selenium.

Hashemi *et al.* (2008) reported that ewes fed at 110% of recommended NRC requirements late gestation produced significantly ($P < 0.01$) more colostrum than ewes fed at 90% and 100% of NRC recommendations. Sufficient protein is vital in late gestation for lamb growth, mammary gland growth, ewe milk yield and colostrum quality (Ocak *et al.* 2005). O'Doherty and Crosby (1997) found that providing a protein source such as soya-bean meal in the last few weeks of gestation of ewes increased the colostrum yield within 1 h and 18 h after birth. Also, colostrum yield increased linearly with an increase in crude protein (CP) intake. Conversely, Ocak *et al.* (2005) found the total colostrum yield was reduced for single bearing ewes supplemented with 1.4 times the protein requirement level for pregnant ewes (165 g CP and 10.5 MJ ME/kg DM) compared to pregnant ewes fed protein at maintenance level. Supplementing single bearing Border Leicester x Merino ewes with lupins and sunflower seed meal from day 130 of gestation until parturition was found to increase colostrum yield (Hall *et al.* 1992).

During gestation, mammary gland growth and development is vital for subsequent lactation (Meyer *et al.* 2011). In ewes, 98% of mammary gland growth occurs during pregnancy, with only 2% of growth occurring during lactation. Any changes in the absolute or proportional size of the mammary gland can reduce the amount of colostrum produced (Swanson *et al*. 2008). Mammary gland growth can be altered through changes in the nutritional status of dams during gestation (Po *et al.* 2012).

Increasing dietary energy has been observed to enhance mammogenesis in ewe lambs (Swanson *et al*. 2008). Conversely, Mellor and Murray (1985) found ewes that had a reduced nutrient intake in late gestation had lower udder measurements and colostrum yield. The effects of under-nutrition on mammary gland development is very rapid; Meyer *et al.* (2011) reported that mammary gland growth of ewes was impaired within 3 d of nutrient restriction during late gestation.

Length of gestation can also affect the composition of colostrum. Goats with a gestation length of 146 d were found to have a lower colostral IgG concentration than goats with a longer gestation length. This finding was similar for ewes; ewes that had a longer gestation period (150 d) produced 127.7 mg IgG/mL colostrum compared to 99.9 mg IgG/mL for ewes that had a shorter gestation period (146.9 d) (Castro *et al.* 2011).

There are reported differences between primiparous and multiparous ewes in terms of colostral IgG concentration. In primiparous ewes it is thought that the higher colostral IgG concentration is due to a lower colostrum volume (Tabatabaei *et al.* 2013). A reduced colostrum output was observed for multiple bearing ewes compared to single bearing ewes, although supplementation with sunflower seed meal of twin-bearing ewes increased their colostrum yield (Hall *et al.* 1992). Nowak and Poindron (2006) reported twin-bearing ewes generally yield more colostrum than single-bearing ewes; however, the onset of lactation is slower and less colostrum is produced per lamb.

2.2.2 Assessing colostrum quality

The quality of colostrum is based on the concentration of IgG (Wereme *et al.* 2001; Tabatabaei *et al.* 2013; Yilmaz and Kasikci 2013) and several diagnostic tools have been developed to assess colostrum quality. The colostrometer (hydrometer) measures colostral specific gravity (Bielmann *et al.* 2010; Zarrilli *et al.* 2003a). Several factors have been found to influence the results including breed (Morin *et al*. 2001; Quigley *et al.* 2013), season of the year (Quigley *et al.* 2013) and colostral temperature (Mechor *et al*. 1992; Bielmann *et al.* 2010; Quigley *et al.* 2013). Quigley *et al*. (1995) and Fleenor and Stott (1980) found that bovine colostral specific gravity was more strongly correlated with total protein concentration rather than immunoglobulin concentration. This may result in the specific gravity reading providing false information regarding the actual concentration of immunoglobulins in the colostrum.

A hydrometer has also been used to estimate immunoglobulin concentration in caprine colostrum, with only moderate accuracy (Rudovsky *et al.* 2008). Colostrometer is not recommended

for use with ewe colostrum because it requires a relatively large amount of colostrum, around 250 mL (Zarrilli *et al*. 2003a).

Liquid chromatography, reversed-phase liquid chromatography, size exclusion chromatography, ion exchange chromatography and affinity chromatography are all separation-based techniques and have been used to identify and quantify IgG in bovine milk and colostrum in numerous studies. The type of chromatography used depends on the individual proteins of interest and all methods vary in ability to estimate IgG concentration (Gapper *et al.* 2007).

Radial immunodiffusion (RID) is an immune-based technique that can be used to determine colostral IgG concentration in humans, sheep, goats, horses, pigs and cattle. Colostrum samples are applied to wells in an agarose gel with antibody raised to the species IgG. The sample then diffuses through the gel and the IgG forms a precipitin complex with the antibody. The amount of IgG present in the sample is proportional to the ring diameter (Gapper *et al.* 2007).

Brix refractometers can be used to estimate the IgG concentration in the colostrum of sheep, horses and cattle (Bielmann *et al.* 2010; Quigley *et al.* 2013). There are two types of Brix refractometers, optical and digital, and both measure the refractometric index of liquids on a Brix scale. An optical Brix refractometer requires someone to peer into the instrument and determine the Brix percentage by identifying a blue line on the scale. Whereas, a digital Brix refractometer shines a light through the liquid sample to measure the index of refraction and present the reading digitally on a scale in Brix units. A digital Brix refractometer may provide results that are more consistent and accurate because the values are determined digitally, whereas optical Brix refractometer values are open to individual interpretation (Bielmann *et al*. 2010). Brix refractometers, unlike colostrometers, are not sensitive to the temperature of colostrum used for analysis (Bielmann *et al*. 2010; Quigley *et al*. 2013). However, the volume and proportion of protein in colostrum can influence the accuracy of the refractometer. Brix refractometers are highly correlated with RID analysis of IgG, are inexpensive, simple to use and durable. Various studies (Bielmann *et al*. 2010; Quigley *et al*. 2013) have determined that the optimal cut-off value for Brix refractometer is equal to or above 21% to ensure high quality colostrum with > 50 mg IgG/mL. At the 21% cut-off point, sensitivity and specificity is increased and the Ig concentrations in colostrum can be correctly determined with minimal false negatives.

Dry chemistry techniques are used for colostral enzyme assays to find potential markers suitable for determining colostrum quality. The colostral enzymes gamma glutamyltrasnferase (GGT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) have been used to determine colostrum quality in ewes (Zarrilli *et al.* 2003a) and goats (Zarrilli *et al.* 2003b). Colostrum proteins are separated

using electrophoresis and then the quality of colostrum is determined from the content of IgG (Maden *et al.* 2003). Zarrilli *et al.* (2003a) found the GGT enzyme could be a potential marker for the evaluation of colostrum quality in ewes because it was highly correlated with IgG concentration and had the highest activity, followed by LDH and then ALP. It was hypothesised that GGT is found at higher concentrations in ewes colostrum compared to LDH and ALP due to GGT being involved in the process of colostrum synthesis. Maden *et al*. (2003) found that serum GGT activity had a test sensitivity value of 100% and 68% positive predictions for IgG concentration in colostrum and milk, respectively.

2.3 Passive immunity

Lambs and calves are born agammaglobulinaemic (Wereme *et al*. 2001; Maden *et al.* 2003; Yilmaz and Kasikci 2013) as ruminants have an epitheliochorial placenta (Pattinson *et al*. 1995; Sammin *et al.* 2009) whereby the foetal chorionic epithelium is in contact with the intact uterine epithelium. In this type of placentation, trans-placental passage of immunoglobulins is prevented (Lazarevic *et al*. 2010; Yilmaz *et al*. 2011; Dudek *et al*. 2014) as there is a syncytium between the maternal endometrium and the foetal trophectoderm, thus separating the maternal and foetal blood supply (Weaver *et al.* 2000; Sammin *et al*. 2009).

The ovine foetus usually develops without exposure to foreign antigenic material. However, if the ovine foetus is subjected to an antigen challenge *in utero* it is capable of activating specific antibodies. During a ewe's pregnancy endometrial intraepithelial lymphocytes (IEL) that express the $\gamma\delta$T-Cell receptor are thought to suppress the anti-foetal immune responsiveness. At parturition, there is a decline in IEL due to degranulation and apoptosis (Sammin *et al.* 2009).

The newborn utilises the process of pinocytosis to non-selectively absorb intact large protein molecules, such as immunoglobulins (Brujeni *et al*. 2010; Hine *et al.* 2010; Yilmaz and Kasikci 2013) via neonatal enterocytes in the small intestines (Weaver *et al.* 2000). Pinocytosis involves cells ingesting extracellular fluid and its contents (Broughton and Leece 1970). This process leads to immunoglobulins being transported across cells and released into the lymphatics by exocytosis. After this stage they enter the circulatory system through the thoracic duct (Dominguez *et al*. 2001; Wereme *et al*. 2001; Zarrilli *et al*. 2003a). Newborn ruminants require a sufficient amount of colostrum within 48 h post-partum (Stelwagen *et al.* 2009; Hernandez-Castellano *et al.* 2014) because the enterocytes of the small intestine lose their ability to absorb antibodies within 24 - 48 h post-partum (Weaver *et al.* 2000; Sangild 2003). Thus, there is a restricted time period for the transition of immunoglobulins into the intestines of newborns (Hart *et al.* 2009; Yilmaz and Kasikci 2013). Intestinal closure can be species

specific, and closure may occur more rapidly or be extended depending on feeding regimens. Intestinal closure in lambs is reported to occur at around 24 h after birth (Nowak and Poindron 2006). In calves, the first 4 h post-partum is the optimal time for the transfer of immunoglobulins across the gut epithelium. After 12 h post-partum immunoglobulin transfer begins to decline (Weaver *et al.* 2000; Morrill *et al.* 2013). Morin *et al.* (1997) found prior to closure the amount of colostral immunoglobulins absorbed increased as the amount of high quality colostrum fed to Holstein bull calves increased. After the absorption of maternal immunoglobulins and intestinal closure, the concentration of immunoglobulins in (lamb) serum declines as they are catabolised (Dominguez *et al.* 2001).

The absorption of IgG is vital for the immune protection of neonates; IgG acts by binding to specific sites on the surfaces of infectious agents or products, and is involved in complement activation and bacterial opsonisation and agglutination (Gapper *et al.* 2007). It is difficult to precisely define the concentration of immunoglobulin in the blood that provides satisfactory protection to the lamb or calf. The concentration of IgG in serum needed for protection in calves varies between studies; however, generally failure of passive transfer is considered to be when serum Ig concentrations are below 10 mg/mL (Jaster 2005, Porto *et al.* 2007; Furman-Fratczak *et al.* 2011). A serum IgG concentration for lambs above 10 mg/mL is thought to be adequate for lamb survival (Britti *et al.* 2005).However, lambs have been found to have a failure in passive transfer when the concentration of IgG in their serum was < 6 mg/mL (McGuire *et al.* 1983). Boland *et al.* (2005) reported a wide range in the concentration of IgG in lamb serum, from 4.2 mg/mL to 20.9 mg/mL.

The variation in neonatal serum immunoglobulin concentration is often associated with morbidity and mortality and inversely related to disease prevalence (Yilmaz *et al.* 2011). The concentration of immunoglobulins in the serum of the newborn is lowest 1 h post-partum; however, after successful transfer of passive immunity in young animals, it increases significantly 24 h post-partum (Hashemi *et al*. 2008).

Any delay in adequate suckling by the lamb, either due to poor teat-seeking activity or low colostrum yield, can reduce the chance of successful passive transfer (Nowak and Poindron 2006). Lambs that have a low birth weight tend to have a lower serum IgG concentration compared to lambs with a heavier birth weight (Yilmaz *et al.* 2011). Low birth weight and low serum immunoglobulin concentration increases the risk of lamb mortality (Christley *et al.* 2003) because such lambs tend to have a low energy reserve and are weaker (Nowak and Poindron 2006). If neonatal vigour is adequate then the ability of the gastro-intestinal tract (GIT) of neonate to absorb IgG and nutrients and the

ability of the dam's mammary gland to provide suitable nutrients via milk will determine the immune status of the neonate and its ability to survive (Swanson *et al.* 2008).

Ruminants have primary and secondary lymphoid tissues that contain immune cells and B-lymphocytes. On exposure to antigens the B-lymphocytes synthesise immunoglobulins. Hence, cells from lymphoid tissues, along with neutrophils and passively acquired IgG from colostrum all interact together to provide immunity to young animals (Murphy *et al.* 2014).

2.3.1 Factors affecting absorption of colostral immunoglobulins

The efficiency of colostral immunoglobulin absorption through the intestinal epithelium decreases linearly with time from birth to 24 h of age (Weaver *et al*. 2000). This is due to the maturation of intestinal epithelial cells (Jochims *et al*. 1994) and the secretion of digestive enzymes. For a limited period postpartum the secretion of digestive enzymes in the neonate is postponed to allow the macromolecules to pass through the glandular stomach and be absorbed (Thivend *et al*. 1980). The efficiency of IgG transfer across the small intestine epithelium is optimal 4 h postpartum, and after 6 h it begins to steadily decline (Besser *et al.* 1985). After approximately 12 h enzyme secretion increases thus reducing the ability of IgG to reach the small intestine without being degraded (Quigley *et al*. 1995). For these reasons it is critical that the timing of the first maternal colostrum feeding is appropriate in order to ensure successful transfer of immunity (Yilmaz and Kasikci 2013). Weaver *et al.* (2000) found that calves fed colostrum earlier had significantly higher serum IgG concentrations than calves fed later with similar (IgG) concentrations and volumes of colostrum.

Premature birth has been associated with decreased ability of newborns to absorb immunoglobulins from colostrum. This is because intestinal enterocytes do not reach their full endocytotic potential, which is due to the lack of the specific maturational process that occurs close to parturition to allow newborns to take up and transfer intact proteins (Sangild 2003). Birthing difficulties, such as dystocia in calves has been found to cause a reduction in IgG absorption (Jacobsen *et al.* 2002; Sangild 2003). However, there is limited understanding as to how dystocia can limit IgG absorption (Sangild 2003).

Intestinal closure can have an effect on immunoglobulin absorption; the average time of intestinal closure for IgG, IgM and IgA is 26.4 h, 25 h and 26 h, respectively. The time colostrum is initially taken by the newborn is thought to influence intestinal closure (Yilmaz and Kasikci 2013) and therefore the level of antibodies absorbed. The ability for lambs to absorb colostral immunoglobulins lasts between 24 to 36 h post-partum, before intestinal closure and activation of the neonate's digestive system (Dominguez *et al.* 2001).

Breed may also have an effect on the absorption of immunoglobulins; IgG_1 concentrations (at 24 to 48 h of age) have been found to be higher in Angus compared to Simmental (Engle *et al*. 1999), Hereford and Red Poll (Muggli *et al*. 1987) calves. Differences in IgG profiles have been observed in different breeds of lambs. Brujeni *et al*. (2010) found that at day 1 post-partum the serum IgG concentration of Shaul lambs was 32.1 mg/mL and by day 15 had decreased to 22 mg/mL, compared to Finnish x Dorset Horn lambs that had serum IgG concentrations of 22 mg/mL and 12 mg/mL at 1 and 14 d post-partum, respectively. In comparison, Waelchli *et al*. (1994) reported Lacaune and Ostfriesisches milchschaf lambs had an initial IgG concentration of 30.2 mg/mL and 41.3 mg/mL, respectively and then by day 14 the concentrations had decreased to 17.8 mg/mL and 15 mg/mL, respectively.

Gender may also influence immune status. Ahmad *et al.* (2000) found that male lambs had higher albumin and globulin concentrations than female lambs whilst Brujeni *et al*. (2010) reported there was no interaction between sex of lambs, serum IgG and total immunoglobulin concentrations. Thompson *et al.* (2013) also found there was no association between the sex of Springbok calves and colostrum absorption or IgG concentrations.

The nutritional status of the dam can influence the intestinal permeability of newborn lambs to immunoglobulins (Hodgson *et al*. 1997). Newborn lambs have been found to have greater absorption of immunoglobulins when the dam is healthier. Furthermore, nutritional supplementation of dams can enhance the erythropoietic response and therefore improve offspring survival (Ahmad *et al*. 2000). Rock *et al.* (2001) found lambs born to ewes that were selenium supplemented during gestation had a higher serum IgG concentration than lambs born to non-supplemented ewes. Selenium supplementation increases colostral IgG which enhances the ability of both calves and lambs to absorb IgG, therefore increasing serum IgG concentrations (Rock *et al.* 2001).

2.3.2 Failure of passive immunity transfer

Failure of passive transfer (FPT) predisposes the neonate to disease (Brujeni *et al*. 2010; Vandeputte *et al.* 2011) due to them being hypogammaglobulinaemic (Britti *et al.* 2005; Mech *et al.* 2011). Low absorption of immunoglobulins often results in an increase in diseases such as diarrhoea, enteritis, septicaemia, arthritis, pneumonia (Thompson *et al.* 2013) and high risk of mortality (Ahmad *et al.* 2000; Wereme *et al.* 2001; Zarrilli *et al.* 2003a; Stelwagen *et al.* 2009; Furman-Fratczak *et al.* 2011). Failure to acquire passive immunity can be directly responsible for half of all neonatal mortalities (Murphy *et al.* 2014). Factors influencing colostral IgG transfer include the quantity of IgG ingested, time period between birth and colostrum intake, the feeding method of colostrum (i.e. via a bottle or

teat) and also genetic, physiological and environmental factors (Wereme *et al.* 2001; Thompson *et al.* 2013). Serum IgG concentration in the ruminant newborn is an important indicator of FPT (Yalcin *et al.* 2010). The prevalence of FPT in lambs has been reported to range from 3.4% to 20%, with mortality rates of 45% to 50% in lambs from birth up until they are two to three weeks of age (Britti *et al.* 2005).

A circulating IgG concentration of < 10 mg/mL at 24 h (Morrill *et al.* 2013; Quigley *et al.* 2013; Deelen *et al.* 2014) or 48 to 72 h post-partum (Thompson *et al.* 2013) has been defined as FPT in calves. Calves with serum IgG concentrations of IgG < 10 mg/mL have been found to have an increased risk of mortality (Britti *et al.* 2005). Furman-Fratczak *et al.* (2011) found heifer calves with a serum Ig concentration exceeding 10 mg/mL at 30 to 60 h post-partum, had reduced morbidity and did not become ill before 14 d of age. DeNise *et al*. (1988) found that calves with FPT had a far greater mortality rate during the first 180 d of life; 44% of all mortalities during the study had serum concentrations of < 12.1 mg/mL whilst the mortality rate for calves with serum IgG concentrations of > 40 mg/mL was 2.59%. Weaver *et al.* (2000) found that feeding calves greater than 100 g of colostral IgG reduced the rate of FPT. Morrill *et al.* (2013) analysed 150 calf serum samples, and found that 25% of the sampled calves had FPT as indicated by serum IgG concentration of < 10 mg/mL.

Timing of colostrum feeding has a profound effect on the transfer and absorption of colostral immunoglobulins (Morin *et al.* 1997). Multiple births, mastitis, low birth weights, breed, parity and inadequate production of colostrum are all factors that can impact on the newborn's access to colostrum (Wereme et al. 2001). Chirstley *et al.* (2003) found there was a linear relationship between the birth weight of lambs and their serum immunoglobulin status. Newborns that have a lower birth weight tend to be weaker and therefore are unable to or have more trouble suckling adequate amounts of colostrum to achieve adequate serum IgG concentrations for initial immune protection (Ahmad *et al*. 2000; Hashemi *et al*. 2008). Therefore, mortality rates are generally higher in newborns with low birth weights. Brujeni *et al.* (2010) found lambs with birth weights lower than 3 kg had significantly lower IgG concentrations than lambs that weighed greater than 3 kg at birth.

The serum IgG concentration for FPT in lambs is yet to be clearly defined, although Massimini *et al.* (2006) suggested the optimal threshold IgG concentration should be between 6 and 16 mg/mL. Nowak and Poindron (2006) recommended that in the first 18 h post-partum, lambs require 180-290 mL of colostrum per kg of body weight for survival. Lambs with serum IgG concentrations < 10 mg/mL are more at risk of FPT and have an increased mortality rate (Britti *et al.* 2005). Gilbert *et al.* (1988) found that mortality was three to four times higher in lambs that had serum IgG concentrations of < 10 mg/mL. McGuire *et al.* (1983) reported a 45% mortality rate in 1-1.5 d old lambs with a serum IgG concentration < 6 mg/mL.

It is unclear as to what maternal and neonatal factors may affect the transfer of passive immunity in sheep and what impact breed may have. Brujeni *et al.* (2010) found that in the fat-tailed Shaul sheep passive immunity transfer was not influenced by sex, litter type and birth weight.

2.3.3 Testing for the transfer of passive immunity

There are many tests available for the testing of passive immunity transfer; however, there are only two quantitative passive immune transfer tests (Thompson *et al.* 2013) that directly measure IgG concentrations and these are radial immunodiffusion (RID) and enzyme-linked immunosorbent assay (ELISA) (Weaver *et al.* 2000). Both RID and ELISA are species specific; they have been found to have a 94% agreement in relation to serum IgG concentrations (Thompson *et al.* 2013). The other tests, which include refractometry, zinc sulfate turbidity test, sodium sulfite turbidity test, serum GGT activity and whole blood glutaraldehyde gelation, measure serum total solids (Weaver *et al.* 2000; Britti *et al.* 2005) and do not quantitatively measure serum IgG concentration (Lee *et al.* 2008).

2.3.3.1 Radial immunodiffusion

Radial immunodiffusion (RID) involves samples being applied to wells in an agarose gel that contains an antibody raised to IgG for a particular species. The sample being analysed then diffuses through the gel and forms a precipitin ring complex with the antibody, and it is the diameter of the ring which is proportional to the concentration of IgG present (Gapper *et al.* 2007). RID is considered the 'gold-standard' for measuring serum IgG concentrations (Dawes *et al.* 2002; Morrill *et al.* 2013; Deelen *et al.* 2014) and is a commonly used method for analysis of IgG in newborn's serum (Quigley *et al*. 2013) as it is accurate (Britti *et al.* 2005; Yalcin *et al.* 2010). However, RID can be expensive and time-consuming because it requires 18-24 h for results from laboratory analysis (Lee *et al.* 2008; Yalcin *et al.* 2010; Tabatabaei *et al*. 2013) and discrepancies do exist between RID kits (Gapper *et al.* 2007; Morrill *et al.* 2013). RID technique has been used for IgG analysis in many species, including bovine, ovine, equine, caprine, porcine and human (Gapper *et al.* 2007).

2.3.3.2 Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assays are used to either qualitatively or quantitatively detect antigens. There are three main forms of ELISA, direct, sandwich and inhibition. ELISA relies on the interaction between an antigen and the antibodies raised against the antigen. The antibodies raised against a species IgG will bind to the wells of the ELISA plate, a colour change will result after the addition of antibody-enzyme conjugate and colorimetric measurement is used to detect and quantify

IgG present (Gapper *et al.* 2007). The concentration of IgG present is then determined from a standard curve.

In comparison to RID, ELISA is cheaper, 4.5 times more time-efficient and is capable of measuring a larger number of samples at once. However, ELISA is carried out in laboratory conditions and involves numerous steps, including coating with capture antibody, blocking, conjugation with detection antibody, and enzyme substrate reaction. Dilution of IgG serum samples and serial dilution of standards can be causes of error in ELISA (Lee *et al.* 2008).

2.3.3.3 Refractometer

Refractometry, using a digital or optical refractometer, is an indirect method that can be used to detect passive immunity transfer (Massimini *et al.* 2006; Morrill *et al.* 2013). It is affordable, simple and quick to perform on-farm, although temperature of the solute can influence the index of refraction. Refractometry has been reported to be inaccurate for use with dehydrated lambs (Massimini *et al.* 2006). The refractometer measurement of serum immunoglobulin concentration is based on the refraction index of the serum and its conversion to serum total protein concentration using a known conversion factor. Depending on the type of refractometer used, the serum total protein concentrations can be under-estimated by 2.7-5.0 mg/mL (Vandeputte *et al.* 2011). Deelen *et al.* (2014) found that Brix measurements were highly correlated with serum IgG (r = 0.93) in dairy calves. Using a refractometer to measure serum total protein, Weaver *et al.* (2000) were able to correctly classify, in terms of successful transfer of passive immunity, > 80% of calves. However, Massimini *et al*. (2006) reported that refractometry measurements can account for around 85% of the variation in serum IgG concentrations.

2.3.3.4 Zinc sulfate turbidity test

Qualitative spectrophotometric zinc sulphate turbidity assay (ZST) can be used to measure total immunoglobulin content (Tabatabaei *et al*. 2013) and ZST tests and the thresholds have been well established for lambs (Massimini *et al.* 2006). This assay involves a single dilution that causes the precipitation of proteins resulting in turbidity, which is measured. ZST assays are not specific to detect IgG. Weaver *et al.* (2000) found that only 69% of calves were correctly classified (in terms of successful transfer of passive immunity) using ZST. Tabatabaei *et al*. (2013) found RID did not correlate with ZST, with estimated IgG concentration based on the ZST test being higher than values from RID.

2.3.3.5 Sodium sulfite turbidity test

Sodium sulphite turbidity test (SST) involves serum being mixed with a sodium sulphite solution (Thompson *et al.* 2013). In the past, SST was a three-step semiquanitative test that used 14, 16 and 18% sodium sulfite test solutions (Dawes *et al.* 2002). These test solutions then cause precipitation of high molecular weight proteins, such as immunoglobulins, resulting in turbidity. The turbidity is the measured endpoint of SST. Single dilution assays are more commonly used as they are more reliable. A 14% test solution has a higher serum immunoglobulin concentration than a 16% test solution, which is higher than an 18% test solution. Therefore, to minimise errors and unreliable results an 18% test solution is best for a single dilution procedure (Weaver *et al.* 2000).

2.3.3.6 Glutaraldehyde coagulation test

Glutaraldehyde coagulation test is applicable on-farm to semi-quantitatively analyse serum IgG and assess passive immunity transfer status as it is fast, simple and affordable. Uncharged amino groups on proteins form cross-linkages with aldehyde groups, forming a visible clot that can be attributed to the gelation of gamma globulins (Weaver *et al.* 2000). GCT has been used successfully to assess the serum IgG concentration and thus passive immunity transfer status in newborn goat kids (Yalcin *et al*. 2010) and calves (Lee *et al.* 2008). However, the sensitivity and specificity of GCT can be variable, ranging from 0.41 to 0.00 and 0.85 to 1.00 (respectively).

2.3.3.7 Serum γ-glutamyl transferase activity

Serum γ-glutamyl transferase activity has been used to monitor the transfer of passive immunity in lambs (Massimini *et al.* 2006; Tsiligianni *et al.* 2012) and calves (Maden *et al.* 2003). GGT is an enzyme produced in the mammary gland cells (Weaver *et al.* 2000; Thompson *et al.* 2013) and is concentrated in the colostrum of ruminants. This enzyme can be readily absorbed by ruminant neonates within 24 to 48 h post-partum (Massimini *et al.* 2006). Serum γ-globulin concentration has been found to be significantly correlated in a linear relationship with serum IgG concentration in 1 d old lambs (Massimini *et al*. 2006) and also calves and kids (Thompson *et al.* 2013). Maden *et al.* (2003) reported lambs that ingested colostrum had serum GGT activities that were 140 times higher than healthy adult sheep, and colostral GGT activities 470 times higher than those in adult sheep serum. In support, Weaver *et al.* (2000) reported serum GGT activity was higher in calves compared to adult cows. Serum GGT activity assays are inexpensive, not affected by dehydration (Thompson *et al.* 2013), rapid and thought to be more accurate than refractometry for predicting passive transfer in lambs (Massimini *et al*. 2006; Tsiligianni *et al.* 2012). Maden *et al.* (2003) found serum GGT activity tests had a sensitivity value of 96% and 100% positive prediction values for serum IgG concentration.

2.4 Conclusion

Passive immune transfer plays a great role in protecting neonates from diseases and environmental extremes, and ensures they begin their life without disadvantage. Colostrum quality and quantity, which can be influenced by breed, nutrition and environmental factors can influence the success of immunoglobulin absorption by ruminant newborns. Various management factors by producers are vital to ensuring ruminants perform to full genetic potential and ensure adequate immunoglobulins are produced in the final stages of colostrum production.

Chapter 3. Scientific Paper

Breed and diet effects on ewe colostrum quality, lamb birthweight and the transfer of passive immunity

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3.1 Abstract

Various factors including breed and the health of ewes may impact on the quality of the colostrum they produce and the subsequent transfer of passive immunity to their lambs. In this study the serum IgG concentrations of 12 Merino and 12 Dorper ewes grazing either dual-purpose wheat or canola during late gestation and the IgG concentration of the colostrum they subsequently produced was measured. The birth weight of their lambs was recorded and the lambs' serum IgG concentration 24 h post-partum measured to assess the success of transfer of passive immunity. The serum and colostral IgG concentrations were quantified using a commercially available ovine ELISA assay. Neither breed nor diet had any effect ($P > 0.05$) on ewe serum (2.91 – 14.07 mg/mL) and colostral (16.98 – 319.52 mg/mL) IgG concentrations. The serum IgG concentration of ewes was significantly ($P < 0.05$) associated with their live weight and body condition score one week pre-partum, although their colostral IgG concentration was not ($P > 0.05$). The colostral IgG concentration was also not associated ($P > 0.05$) with serum IgG concentrations in either the ewes or their lambs (0.68 – 19.60 mg/mL), indicating that neither the immune status of the ewe (serum IgG concentration) nor the quality of the colostrum she produces can be used to predict the success (or failure) of transfer of passive immunity to lambs. However, when fitted to a linear model, the interaction of colostral IgG concentration modified by lamb birthweight did significantly affect ($P < 0.05$) lamb serum IgG concentration. Birth type had a significant effect ($P < 0.05$) on lamb birthweight, with single lambs (5.61 ± 0.306 kg) being significantly heavier than twin lambs (4.86 ± 0.139 kg). There was a highly significant ($P < 0.01$) relationship between lamb serum IgG concentration 24 h post-partum and their survival pre-weaning. The average serum IgG concentration of the lambs that survived (36 out of 40) was 10.99 ± 0.69 mg/mL; the average serum concentration of the (four) lambs that died was 2.77 ± 2.08 mg/mL.

Further research is needed to identify the key factors that affect the transfer of passive immunity in lambs.

Keywords: Immune status, immunoglobulin, neonate survival

3.2 Introduction

Lambs are born agammaglobulinaemic (Hart *et al.* 2009) and thus depend entirely on gaining immunity from their mother after birth to establish a functional immune system (Stelwagen *et al.* 2009). This occurs through the ingestion of colostrum, which is a mixture of lacteal secretions and constituents from blood serum, which consist of immunoglobulins and other serum proteins (Foley and Otterby 1978). Colostrum also contains various cellular and humoral factors (Yilmaz and Kasikci 2013), along with insulin-like growth factor (Ocak *et al.* 2005; Nowak and Poindron 2006), insulin, growth hormone, thyroxine, triiodothyronine, and prolactin (Yilmaz and Kasikci 2013) that aid in the survival of newborns.

The neonatal Fc receptor (FcRn) is involved with transferring immunoglobulins from the dam's circulation to the alveolar and ductal cells of the udder to produce colostrum (Baintner 2007; Yilmaz *et al.* 2011; Tabatabaei *et al.* 2013). The colostrum immunoglobulins, mainly IgG, along with the ability of the neonatal gut to allow unrestricted passage of the large immunoglobulin molecules, provide passive immunity to neonates (Gapper *et al.* 2007; Stelwagen *et al.* 2009). Unsuccessful transfer of passive immunity results in an increased risk of mortality and morbidity in the neonatal period (Ameri and Wilkerson 2008; Tabatabaei *et al.* 2013). Low production of colostral IgG is considered one of the major factors contributing to the failure of transfer of passive immunity in lambs (Tabatabaei *et al.* 2013). Colostral IgG concentration, influenced by maternal genetics, varies widely between (Snowder and Glimp 1991) and within breeds (Tabatabaei et al. 2013), and is also modulated by ewe nutrition during late gestation (Boland *et al.* 2005; Swanson *et al.* 2008) and birth type (Gilbert *et al.* 1988).

Any delay in suckling by the lamb, either due to poor teat-seeking activity or low colostrum yield, can reduce the chance of successful passive transfer (Nowak and Poindron 2006). Lambs that have a low birth weight tend to have a lower serum IgG concentration compared to lambs with a heavier birth weight (Yilmaz *et al.* 2011). Low birth weight and low serum immunoglobulin concentration increase the risk of lamb mortality (Christley *et al.* 2003) because they have low energy reserves and are weaker (Nowak and Poindron 2006).

The nutritional status of the dam can influence the intestinal permeability of newborn lambs to immunoglobulins (Hodgson *et al*. 1997). Newborn lambs have been found to have greater

absorption of immunoglobulins when the dam is healthier. Furthermore, nutritional supplementation of dams can enhance the erythropoietic response and therefore improve offspring survival (Ahmad *et al*. 2000).

Numerous studies have been undertaken analysing the effects that diet and breed have on bovine colostrum quality and the transfer of passive immunity in calves. However, limited information exists on potential differences in the immune status and subsequent quality of colostrum produced by ewes. The aim of this study was to determine if the immune status and colostrum quality of Merino and Dorper ewes varied when they were grazed on either dual-purpose wheat or canola forage during late gestation and if this subsequently impacted on the birthweight and/or transfer of passive immunity to their lambs and their survival.

3.3 Materials and method

This study was conducted with approval from the Charles Sturt University Animal Care and Ethics Committee, approved under the 12/101 protocol.

3.3.1 Animals

Twenty four ewes (12 Merino and 12 Dorper; average live weight of 89.28 ± 8.05 kg and BCS 3.7) and their lambs were randomly selected from a larger mob of 142 pregnant ewes (92 Dorper ewes 4-5 years old and 50 Merino ewes 6-7 years old). Of those 142 ewes, 44 Dorper ewes were joined to White Dorper rams, 48 Dorper ewes and all Merino ewes were joined to White Suffolk rams. Ewes were joined with the rams from 17th February until 31st March, 2014. Ewes were pregnancy scanned and foetal aged on 30th April and re-scanned on 15th May, 2014. Throughout joining and the first two-thirds of gestation the ewes grazed pasture consisting mainly of lucerne (*Medicago sativa)* and barley grass (*Hordeum leporinum*). In late gestation ewes were either grazed on dual-purpose wheat (*Triticum aestivum* – EGA Wedgetail) or canola forage (*Brassica napus* -Hyola 971). For the three dual-purpose wheat plots, 1.5 kg of mineral supplement was provided *ad libitum* as a loose lick to the ewes. The supplement consisted of 600 g of magnesium oxide, 600 g of lime and 300 g of salt. The dual-purpose wheat had average nutritive values of 12.98 MJ ME/kg DM, 21% CP and 84.9% DMD and for the canola forage, 14.06 MJ ME/kg DM, 20.3% CP and 91.4% DMD.

3.3.2 Experimental design

This experiment had a randomised block design with a 2 x 2 factorial treatment structure (two diets, two breeds and three replicates).

A 12 ha paddock was divided into six plots (approximately 1.86 ha). Two diet treatments (dual-purpose wheat and canola) were randomly allocated to the six plots earlier in the year. Twenty-two ewes were then randomly allocated to plot 1 (canola), 24 ewes were randomly allocated to plot 2 (dual-purpose wheat), 24 ewes were randomly allocated to plot 3 (dual-purpose wheat), 24 ewes were randomly allocated to plot 4 (canola), 24 ewes were randomly allocated to plot 5 (canola) and 24 ewes were randomly allocated to plot 6 (dual-purpose wheat) to graze late gestation (early July). Two ewes (and their subsequent lambs) of each breed were randomly selected from each plot for sampling of blood and colostrum.

3.3.3 Measurements and sampling

The live weight and body condition scores (BCS) were taken of the ewes, one week prior to lambing and weekly throughout the lambing period.

Within approximately 24 h post-partum blood and colostrum samples were collected from the ewes. Jugular blood samples were taken using venipuncture and were collected using 18 gauge needles and 9 mL vacutubes (VACUETTE® Z Serum Clot activator tubes). Following clot formation the tubes were centrifuged at 3500 rpm for 15 min, the serum collected and stored frozen at -20°C. One side of the udder of each ewe was hand milked and a minimum of 5 mL of colostrum collected and subsequently stored frozen at -20°C.

Shortly after birth, lambs were ear tagged with an identification number and weighed by placing them in a bucket connected to hand scales. Within approximately 24 h post-partum a blood sample was collected from their jugular vein using a needle and syringe and then injected into 9 mL vacutubes (VACUETTE® Z Serum Clot activator tubes). Following clot formation the tubes were centrifuged at 3500 rpm for 15 min, the serum decanted and then stored frozen at -20°C.

The lambs were weighed weekly until the end of the lambing period (which lasted approximately 8 weeks).

3.3.4 Serum and colostral IgG concentrations

A double antibody sandwich ovine IgG enzyme-linked immunosorbent assay, ELISA kit (E-35 G, Immunology Consultants Laboratory) was used to determine serum and colostral IgG concentrations. The methodology was per the manufacturer's protocol; serum samples were diluted 1/400,000 and colostrum samples were diluted 1/100,000,000. The optical density (OD) was set at 450 nm and determined with an automated microplate reader (VersaMax, Molecular Devices).

3.3.5 Statistical analyses

A standard curve for IgG concentration was constructed based upon the standards known concentrations and optical densities, using AssayZap (Version 3.0, Biosoft®). From the standard curve, the optical densities of the samples were interpolated to determine the concentration of the serum and colostrum samples, applying the appropriate dilution factors. The results (serum and colostral IgG concentrations, ewe live weight and BCS, lamb birthweight, birth type, sex and weight gain) were analysed using general linear models and ANOVA fixed effect models for a 2 x 2 factorial design using the statistical program, S+ (TIBCO Spotfire S+ 8.2). P values < 0.05 were considered statistically significant. Model assumptions were tested and models that did not meet Hartley's test for equal variances were weighted.

3.4 Results

3.4.1 IgG concentrations

As shown in Table 3.1, neither breed of the ewe nor the type of pasture they grazed had an effect (P > 0.05) on ewe serum IgG, colostral IgG, or lamb serum IgG concentrations. The average (± SEM) serum IgG concentration of the Merino ewes (8.89 ± 0.779 mg/mL) did not vary (P > 0.05) from that of the Dorper ewes (7.04 ± 0.958 mg/mL). When ewes grazed either dual-purpose wheat or canola their serum IgG concentrations did not differ (P > 0.05; 8.51 ± 0.945 mg/mL and 7.42 ± 0.858, respectively).

Table 3.1. Effect of diet and ewe breed on average ewe serum IgG, colostrum IgG and lamb serum IgG concentrations and lamb birth weight**.**

Breed	Diet	Ewe serum IgG [mg/mL]	Colostrum IgG [mg/mL]	Lamb serum IgG [mg/mL]	Birth weight (kg)
Merino	Wheat	9.05	131.90	11.62	5.55
Dorper	Wheat	7.97	75.69	10.45	4.98
Merino	Canola	8.73	92.32	7.98	4.80
Dorper	Canola	6.11	81.20	10.93	4.79
SEM		1.261	25.75	1.529	0.268

There was a significant (P < 0.05) relationship between serum IgG concentrations of the ewes 24 h post-partum and their live weight one week prior to lambing. There was also a significant (P < 0.01) relationship between the body condition scores of ewes one week pre-partum and their serum IgG concentrations.

Colostral IgG concentrations varied widely (0.19-319.56 mg/mL). The average (± SEM) colostral IgG concentration of the Merino ewes (112.11 ± 23.874 mg/mL) did not vary ($P > 0.05$) from that of the Dorper ewes (78.45 ± 8.313 mg/mL). When ewes grazed either dual-purpose wheat or canola their colostral IgG concentrations did not differ ($P > 0.05$; 103.80 ± 22.593 and 86.76 ± 12.921, respectively). No significant difference ($P > 0.05$) was found in colostral IgG concentrations for ewes that gave birth to a single lamb or twin lambs.

No significant correlation ($r = 0.10$, $P > 0.05$) was found between the serum IgG concentrations of the ewes and the quality (IgG concentration) of the colostrum they produced. There was also no significant ($P > 0.05$) relationship between colostrum IgG concentrations of the ewes 24 h post-partum and either their live weight or BCS one week prior to lambing.

The average (± SEM) serum IgG concentration of the lambs from the Merino ewes (9.62 ± 1.088 mg/mL) did not vary ($P > 0.05$) from that of the lambs from the Dorper ewes (10.71 ± 1.074 mg/mL). When the ewes grazed either dual-purpose wheat or canola the serum IgG concentrations of their lambs did not differ ($P > 0.05$; 11.04 ± 1.086 and 9.45 ± 1.055, respectively). There were no differences ($P > 0.05$) in serum IgG concentrations of the lambs based upon their birth type (singleton or twins), sex or birthweight.

No significant correlation ($r = 0.12$, $P > 0.05$) was found between the quality of the colostrum (IgG concentration) that the lambs consumed and their subsequent serum IgG concentration.

3.4.2 Lamb birth weight

As also shown in Table 3.1, neither the breed of the ewe nor the pasture type they grazed on had any effect ($P > 0.05$) on the birthweight of their lambs. The average birthweight of the lambs born from the Merino ewes was 5.14 ± 0.239 kg, which did not differ ($P > 0.05$) from that of the lambs born from the Dorper ewes (4.88 ± 0.121 kg). When the ewes grazed on dual-purpose wheat the average birthweight of lambs (5.27 ± 0.238 kg) did not differ ($P > 0.05$) from that of the lambs from the ewes that grazed canola (4.80 ± 0.135 kg).

The birth weight of male lambs (5.26 ± 0.219 kg) did not vary ($P > 0.05$) from that of the female lambs (4.78 ± 0.148 kg). However, the birth weight of single lambs (5.61 ± 0.306 kg) was significantly higher ($P < 0.05$) than that of twin lambs (4.86 ± 0.139 kg).

The serum IgG concentration of the dam had no effect ($P > 0.05$) on the birthweight of their lamb(s). The interaction of the serum IgG concentration of the dam and the IgG concentration of the

colostrum they produced also had no effect ($P > 0.05$) on lamb birthweight. However, a significant correlation ($r = 0.36$; $P < 0.05$) was found between colostral IgG concentration and lamb birthweight.

When fitted into a linear model the interaction between colostral IgG concentration and birthweight had a significant effect ($P < 0.05$) on lamb serum IgG concentration. The model for predicting lamb serum IgG concentration based on colostrum IgG concentration modified by lamb birthweight is as follows:

Lamb IgG ~ 18.8632 – 0.1513(Colostral IgG) – 1.1952(Birthweight) + 0.0233 (Colostral IgG x Birthweight)

3.4.3 Lamb survival

The mortality rate of lambs (total of 40 lambs born) in this study was 10%. The four mortalities consisted of one set of twin lambs and two lambs from two different sets of twins. The survival of lambs in the neonatal period was not influenced ($P > 0.05$) by birthweight. However, lamb survival was significantly ($P < 0.01$) related to their serum IgG concentration 24 h post-partum. The average serum IgG concentration of the lambs that survived was 10.99 ± 0.69 mg/mL; the average serum concentration of the (four) lambs that died was 2.77 ± 2.08 mg/mL.

3.5 Discussion

The quality and quantity of colostrum is vital for ensuring lambs acquire passive immunity for survival (Jacobsen *et al.* 2002). Colostral, ewe and lamb serum IgG concentration may be influenced by a number of factors including breed (Gilbert *et al.* 1988; Tabatabaei *et al.* 2013) diet (O'Doherty and Crosby 1997; Fthenakis *et al.* 2012), age (Halliday 1978), genetics (Baintner 2007), litter type (Hart *et al.* 2009; Yilmaz and Kasikci 2013), lamb birth weight (Ahmad *et al.* 2000; Boland *et al.* 2005) and environmental factors (Halliday 1978).

3.5.1 Ewe serum IgG concentrations

There were no significant breed effects on ewe serum IgG concentrations 24 h post-partum. The mean serum IgG concentration was 7.04 ± 0.958 mg/mL for the Dorper ewes and 8.89 ± 0.779 mg/mL for the Merino ewes. This is lower than the values reported for other sheep breeds; Vatankhah (2013) reported serum IgG concentration of 21.33 mg/mL in Lori-Bakhtiari ewes, taken two weeks prior to parturition whilst Hashemi *et al.* (2008) reported serum IgG concentration of 11.6 ± 0.7 mg/mL at 1 h post-partum in Karakul ewes. Further research is needed to establish how ewe serum IgG concentrations vary pre- and post-partum to determine whether these differences in reported IgG concentrations reflect differences in breed or the time relative to parturition that the samples were

collected. In ewes the serum IgG concentration peaks 1-2 months before lambing and then decreases post-partum (Hashemi *et al.* 2008; Hine *et al.* 2010). In cattle, Herr *et al.* (2011) found that serum IgG concentrations began declining in the eighth week ante partum, due to the immunoglobulins going from circulation into the udder tissue. Thus to gain a better insight on the ewe's immune status, and how this may affect IgG concentrations of the colostrum she produces, it may be more appropriate to take blood samples from the ewes in the last few weeks of gestation as opposed to taking them 24 h post-partum as was the case in this study.

The type of pasture the ewes grazed on had no effect ($P > 0.05$) on their serum IgG concentrations (as a measure of their immune status). Hashemi *et al.* (2008) also found there was no significant difference ($P > 0.05$) in the immune status of ewes fed 90%, 100% and 110% of NRC requirements. Daniels *et al.* (2000) reported that oral vitamin E supplementation in late gestation of Targhee twin-bearing ewes did not affect their serum IgG concentrations 4 h post-partum.

The finding that diet had no effect on ewe serum IgG concentration may not hold true for all diets ewes graze, especially diets that vary more in terms of their nutritive value. Future research, into the effects varying quality and types of diets have on the immune status of ewes would be beneficial to further confirm diet is not a source of variation for ewe immune status.

Ewes used in this trial were in excellent condition and ranged from a body condition score (BCS) 3 – 5 (1-5 scale). The live weight of ewes (kg) one week pre-partum was significantly associated with their serum IgG concentrations 24 h post-partum. The BCS of ewes (1-5 scale) was also found to effect ewe serum IgG concentrations. There have been limited reports found in the literature of such effects. Conversely, Vatankhah (2013) found no significant effects of live weight and body condition score on immunity traits, including ewe immune status.

3.5.2 Colostral IgG concentrations

Colostrum IgG concentrations obtained for the sheep used in this study ranged widely, viz. 16.98 – 319.52 mg IgG/mL, which is similar to the range in concentrations reported in other studies in other breeds of sheep (Pattinson *et al.* 1995; Quigley *et al.* 2013; Vantankhah 2013). Colostrum quality (IgG concentration in particular) is influenced by many environmental and genetic factors (Hart *et al.* 2009; Vatankhah 2013). However, in this study neither the breed of the ewe nor the type of pasture they grazed was found to affect colostral IgG concentrations. The average (± SEM) colostral IgG concentration was 112.11 ± 23.874 mg/mL for the Merino ewes and 78.45 ± 8.313 mg/mL for the Dorper ewes. Similarly, in comparing Cheviot, Merino, Finnish Landrace, Finnish x Dorset Horn, Scottish Blackface, Merino x Cheviot and Southdown ewes, Halliday (1978) found no differences in

colostral IgG concentrations. In contrast, Gilbert *et al.* (1988) reported a significant ($P < 0.05$) effect of breed on colostrum quality when comparing Polpay, Rambouillet, Targhee, Columbia, Finnish Landrace and Finne cross ewes. Tabatabaei *et al.* (2013) also found there was a significant breed difference in the colostral IgG concentrations for Shaul and Lori Bakhtyari ewes (62.86 ± 2.48 and 48.82 ± 2.10 mg IgG/mL, respectively). Pattinson *et al.* (1995) found that serum IgG concentration in mature Suffolk x Cambridge ewes was 116 mg/mL at 1 h post-partum and by 24 h post-partum had declined to 15 mg/mL. The findings of this study and comparison to other published results indicate there are wide variations between and within breeds. Hence, more studies need to be conducted to determine if breed differences truly exist or there are other factors involved, not just maternal genetics. Polymorphism in the neonatal Fc receptor gene, which is involved in transferring IgG from serum to colostrum (Yilmaz *et al.* 2011), has been suggested as the source of inter-breed variation in colostrum quality (Tabatabaei *et al.* 2013). Investigation of the neonatal Fc receptor gene in Merinos and Dorpers may shed some light as to why there were no breed differences in this instance.

The pasture type the ewes grazed on had no effect on colostral IgG concentrations supporting the findings of Tabatabaei *et al.* (2013). Hashemi *et al.* (2008) similarly found the quality of the diet that Karakul ewes were fed in late gestation did not affect colostral IgG concentrations. Protein supplementation of ewes consuming either silage or beet pulp has also been found to have no effect on colostral IgG concentrations (O'Doherty and Crosby 1997). Supplementation with fish oil has been found to adversely affect colostral IgG concentrations; Annett *et al.* (2009) found that feeding fish oil to Texel x Greyface ewes in late gestation resulted in them producing colostrum with lower IgG concentrations compared to non-supplemented ewes. Also, large variations in colostral IgG concentration have been reported for ewes supplemented with various mineral/vitamin supplements; ewes supplemented with the Zn treatment (calcium, phosphorous, magnesium, sodium, selenium, cobalt, manganese, iodine and vitamin E) were found to have the highest immunoglobulin concentration 1 h post-partum compared to ewes in the Co treatment (calcium, phosphorous, magnesium, sodium, selenium, zinc , manganese, iodine and vitamin E) and then at 10 h compared to ewes not supplemented with any minerals or vitamins and also ewes supplemented with the I treatment (calcium, phosphorous, magnesium, sodium, selenium, zinc, manganese, cobalt and vitamin E) (Boland *et al.* 2005).

No significant difference ($P > 0.05$) was found in colostral IgG concentrations for ewes that gave birth to a single lamb or twin lambs, which supports the findings of Al-Sabbagh (1995), Pattinson *et al.* (1995) and Tabatabaei *et al.* (2013). This is in contrast to Gilbert *et al.* (1988) who found a significant ($P < 0.01$) linear increase in colostral IgG when litter size increased (mean colostral IgG for singles was 61 mg IgG/mL, 69 mg IgG/mL for twins and 77 mg IgG/mL for triplets).

IgG concentration in colostrum is known to peak 1 h post-partum and then significantly decrease at 12 and 24 h post-partum (Pattinson *et al.* 1995; Hashemi *et al.* 2008), although colostrum can be secreted by the mammary gland for the first few days following birth (Galan-Malo *et al*. 2014). Boland *et al.* (2005) found the mean colostral IgG in mixed breeds to decline over 18 h from birth, from 89 mg IgG/mL 1 h post-partum to 57 mg IgG/mL 10 h post-partum and reach 24 mg IgG/mL at 18 h post-partum. Colostrum samples were collected 24 h after the ewe was noted to have given birth on lambing rounds. This is a potential source of variation in colostrum quality because some of the colostrum samples may have been taken more than 24 h post-partum if the ewes lambed several hours prior to the timing of lambing rounds. Pattinson *et al.* (1995) found as time increased from 1 h to 18 h post-partum the variability in the concentration of IgG in colostrum increased. Also, as some of ewes sampled in this study gave birth to twins, the lambs may have consumed the majority of the colostrum at peak IgG concentration before the colostrum sample was taken. Hart *et al.* (2009) reported immunoglobulin concentration in colostrum was greatest at a lamb's first feed; however, it rapidly declined following subsequent feeds and by the fifth feed only 6% of the original Ig concentration at the first feed was available.

No correlation was found between ewe serum IgG concentration and colostral IgG concentration, which is in contrast to Vatankhah (2013) who reported a significant correlation between ewe serum IgG and colostral IgG concentration. Hashemi *et al.* (2008) similarly reported that the immune status (IgG concentration) of ewes significantly influenced the quality of colostrum they produced. However, Tabatabaei *et al.* (2013), who also found no correlation between ewe serum and colostral IgG concentration, concluded that it would be inaccurate to use ewe serum IgG concentrations to predict the concentration of IgG in colostrum.

The colostrum IgG concentration of ewes 24 h post-partum was not to be affected by their live weight or body condition score one week pre-partum. In support, Al-Sabbagh *et al.* (1995) also found ewe body condition score at lambing did not affect colostral IgG concentration. There are no reports in the literature on the effect of ewe live weight pre-partum on colostral IgG concentration.

3.5.3 Lamb birthweight

Lamb birth weight can have a major influence on lamb survival post-partum (Nowak and Poindron 2006). The energy intake of ewes throughout late gestation influences the birth weight of lambs, with an increase in energy intake positively affecting lamb birth weight (Khalaf *et al.* 1979; Gardner *et al.* 2007). Boland *et al.* (2005) found lamb birthweight was not affected by mineral supplementation (calcium, phosphorous, magnesium, sodium, selenium, cobalt, manganese, iodine, zinc) of ewes late

gestation. No significant effect of diet or breed on lamb birth weight (P > 0.05) was found in this study-. This was not an unexpected finding given that the two pasture types the ewes grazed on during late gestation were of high quality and thus energy intake was unlikely to have differed for ewes grazing the different pastures.

Birth type (single or twins) had a significant effect (P < 0.05) on the birthweight of lambs, with single lambs being heavier (5.61 ± 0.306 kg) than twin lambs (4.86 ± 0.139 kg). This finding is supported by various reported literature (Khalaf 1979; Fthenakis *et al.* 2012). No significant (P > 0.05) difference in the birthweights of male and female lambs was found, and this was supported by Brujeni *et al.* (2010). However, Ahmad *et al.* (2000) reported that male lambs were significantly heavier than female lambs.

3.5.4 Lamb serum IgG concentrations

Lamb serum IgG concentrations 24 h post-partum ranged from 0.68-19.60 mg IgG/mL. This is consistent with the considerable variability in lamb serum IgG concentrations reported by Boland *et al.* (2005), Hashemi *et al.* (2008) and Vatankhah (2013).

Lamb serum IgG concentration was not influenced (P> 0.05) by ewe breed or diet, birth type, birthweight, sex or colostral IgG concentration. Halliday (1974) also found no breed differences in the IgG serum concentration between Merino and Scottish Blackface lambs. However, Gilbert *et al.* (1988) reported significant breed differences in serum IgG concentration of lambs from Polpay, Rambouillet, Targhee, Columbia, Finnish Landrace or Finne cross ewes.

Maternal diet has been found to have variable effects on the IgG concentrations of the lamb. Hashemi *et al.* (2008) fed ewes at 90%, 100% and 110% of NRC requirements during late gestation and found this had no effect on the subsequent IgG concentrations of their lambs. Daniels *et al.* (2000) reported that Vitamin E supplementation of ewes in late gestation also had no effect on the subsequent IgG concentrations of their lambs. Boland *et al.* (2005) fed various mineral supplements to ewes during late gestation and found that the lambs born to ewes supplemented with iodine, along with magnesium, zinc, phosphorous, selenium, manganese and cobalt had lower serum IgG concentrations than lambs from ewes supplemented with the same minerals, excluding iodine and ewes supplemented with no minerals. Providing a fish oil supplementation to ewes during late gestation has also been found to decrease serum IgG concentrations in their lambs (Annett *et al*., 2009).

Sex of the lamb had no effect on serum IgG concentrations which is consistent with the findings of Halliday (1978), Gilbert *et al.* (1988), Brujeni *et al.* (2010) and Vatankhah (2013). Birth type also did not affect lamb serum IgG concentration, which is similar to the findings of Brujeni *et al.* (2010) In contrast, Halliday (1974; 1978) and Gilbert *et al.* (1988) reported birth type to be a source of variation ($P < 0.05$) in lamb serum IgG. They found that as litter size increased, the lamb serum IgG concentration decreased.

The immune status (serum IgG concentration) of the lamb was not related to the quality (IgG concentration) of the colostrum they consumed, which supports the findings of Halliday (1974) and Brujeni *et al.* (2010). In studies with cattle, Garner (2013) also found no correlation between colostral IgG concentration and calf serum IgG concentration. In contrast, a positive correlation between colostral IgG and serum IgG concentrations has been reported in lambs (Nowak and Poindron 2006; Vantankhah 2013) and calves (Jacobsen *et al.* 2002).

Birth weight was also found to have no effect on lamb serum IgG concentration, which is consistent with the findings of Halliday (1974), Gilbert *et al.* (1988) and Brujeni *et al.* (2010). In contrast, Kaymaz *et al.* (2000), Khan *et al.* (2006) and Yilmaz *et al.* (2011) found that lamb IgG concentrations were positively correlated with lamb birth weight.

Although, there were no main effects of colostrum quality or birth weight on lamb immune status, the interaction of birth weight and quality of colostrum consumed had a significant ($P < 0.05$) effect on lamb serum IgG concentration. Therefore, from the birthweight of the lambs in this study modified by the quality of colostrum they consumed their serum IgG concentration could be influenced. Boland *et al.* (2005) suggested that lamb serum IgG was a function of not only lamb birth weight, but also the total IgG consumed and the efficiency of colostral IgG absorption. Ewes may produce high quality colostrum, however they need to produce a high volume of it and the lamb has to suckle to absorb the maternal immunoglobulins, mainly IgG. From this study, if the lamb does not suckle (due to low birth weight) and only consumes poor quality colostrum it could be predicted that the lamb will have a lower serum IgG concentration because they have not adequately acquired passive immunity.

The intake of IgG by the lamb is influenced not only by the IgG concentration but also the quantity of colostrum consumed (Hart *et al.* 2009; Quigley *et al.* 2013). Shubber *et al.* (1979) found there was a strong correlation between the quantity of colostrum consumed and the immune status of lambs 30 h after that feed. Analysing colostrum quantity, which was not a focus of this study, along

with colostrum quality would be beneficial to establish if the interaction of the two influences the transfer of passive immunity to lambs.

3.5.5 Lamb survival

More than 80% of lamb losses occur within 48 h of life (Hinch and Brien 2014). Failure of transfer of passive immunity is associated with increased risks of morbidity and mortality, lower growth rates and poor productivity (Alley *et al.* 2012). Around 14% of lamb mortalities, due to failure or inadequate transfer of passive immunity, occurs between 2- 10 days of age and 5% after 10 days of age (Hart *et al.* 2009).

To meet immunological and energy requirements for survival in their first few days of life, it is recommended that neonates consume more than 112 mL colostrum/kg birth weight in the first 24 h after birth (Boland *et al.* 2005). Lambs with low birth weights (< 3 kg) tend to take longer to stand and suckle (Ahmad *et al.* 2000; Nowak and Poindron 2006), often resulting in low serum IgG concentrations, leading to increased lamb mortality (Christley *et al.* 2003). All of the lambs in the present study were heavier than 3 kg, which has previously been described as the critical birthweight for lamb survival (O'Doherty *et al.* 1998), and explains why no link was found between birth weight and lamb survival in this study. However, a highly significant relationship ($P < 0.01$) was found between serum IgG concentrations and lamb survival. The average serum IgG concentration of the lambs that survived was 10.99 ± 0.69 mg/mL and the average serum concentration of the (four) lambs that died was 2.77 ± 2.08 mg/mL. Halliday (1974) and Tabatabaei *et al.* (2013) also found lambs that died had lower serum IgG concentrations than lambs that survived. Vatankhah (2013) reported mortality rates of 67% in lambs deprived of colostrum compared to 13% in lambs that consumed colostrum.

Based on probability analysis with 95% confidence, lambs with serum IgG concentrations 24 h post-partum of < 7 mg/mL had failure of transfer of passive immunity (and died) and lambs with IgG concentrations > 10 mg/mL had successful transfer of passive immunity (and survived). This is consistent with other reports (Khalaf *et al.* 1979; Gilbert *et al.* 1988) of < 10 mg IgG/mL for failure of or inadequate transfer of passive immunity. Gilbert *et al.* (1988) found mortality was three to four times higher in lambs with serum IgG concentrations of < 10 mg/mL. Khalaf *et al.* (1979) found lambs that survived beyond 10 days had a serum IgG concentration of > 20 mg/mL at 24 h of age, compared to lambs that died which had < 18 mg /mL at 24 h of age. Calves are considered to have failure of passive transfer when their IgG concentrations are below 8 mg/mL (Jacobsen *et al.* 2002) and 10 mg/mL (Quigley *et al.* 2003; Ameri and Wilkerson 2008; Alley *et al.* 2012; Morrill *et al.* 2012). Failure of passive

transfer in goat kids has been defined as a serum IgG concentration of < 12 mg/mL (O'Brien and Sherman 1993).

3.6 Conclusion

The breed genotype (Merino and Dorper) and diet (dual-purpose wheat and canola) did not affect the IgG concentration in ewe serum, colostrum and lamb serum; nor did these two experimental treatments influence lamb birthweight. The serum IgG concentration of ewes 24 h post-partum was associated with their live weight and body condition score one week pre-partum; however, their colostral IgG concentration was not. The findings of this study show that serum IgG concentration of Merino and Dorper ewes 24 h post-partum cannot be used to predict the quality of their colostrum 24 h post-partum. Furthermore, colostrum quality or lamb birth weight alone cannot be used to predict the immune status of lambs. The results indicate there are factors other than just the interaction of lamb birth weight modified by the quality of colostrum they consume which determines the successful transfer of passive immunity in lambs. Other factors may include season, genetics, vigour at birth and IgG absorption ability; however, more studies are required to investigate this.

It can be established that lambs with serum IgG concentrations of < 7 mg IgG/mL will have complete failure of transfer of passive immunity, likely resulting in death. Serum IgG concentrations < 10 but > 7 mg IgG/mL, will be termed partial failure of transfer of passive immunity and result in unhealthy lambs more prone to disease. Finally, successful transfer of passive immunity will occur if lambs have a serum IgG concentration > 10 mg/mL. These lambs with successful transfer of passive immunity are more likely to survive the pre-weaning period and then be productive animals throughout their lifetime.

Lamb survival relies on the concentration of IgG in lamb serum; however it is still unclear what other factors could be modified to increase the concentration of IgG in lamb serum and further studies are required to determine this.

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Appendix A : Guide to Authors – *Animal Production Science*

Title
The title should be concise and informative and contain all keywords necessary to facilitate retrieval by modern searching techniques. Additional keywords not already contained in the title or abstract may be listed beneath the abstract. A short title of less than 50 letter spaces, to be used as a running head at the top of the printed page, should be supplied. The title, author(s), address(es) and short title should comprise a separate title page.

Summary text for the Table of Contents
This is a three-sentence paragraph of 50 to 80 words written for interested non-experts, such as journalists, teachers, government workers, etc. The text should be free from scientific jargon, and written at the level of an article in a science magazine. Your first sentence should engage the reader, convincing them that this is an important area. The second sentence should introduce the problem addressed in the paper, and state your main discovery. The final sentence should describe how the results fit into the bigger picture (i.e. implications or impact of the discovery).

Abstract
The abstract (preferably less than 250 words) should state concisely the scope of the work and the principal findings and should not just recapitulate the results. It should be complete enough for direct use by abstracting services. Acronyms and references should be avoided.

Please suggest 3-6 keywords, noting that all words in the title and abstract are already considered to be keywords. Keyword should list alternative spellings, e.g. defense for defence, aluminum for aluminium etc.

References
References are cited by the author and date (Harvard system); they are not numbered. All references in the text must be listed at the end of the paper, with the names of authors arranged alphabetically; all entries in this list must correspond to references in the text. In the text, the names of 2 co-authors are linked by ´and´; for 3 or more, the first author´s name is followed by ´*et al.*´. Where more than one reference is cited in the text, they should be listed chronologically. No editorial responsibility can be taken for the accuracy of the references. The titles of papers and the first and last page numbers must be included for all references. Papers that have not been accepted for publication cannot be included in the list of references and must be cited in the text as ´unpublished data´ or ´personal communication´; the use of such citations is discouraged. Authors should refer to the latest issues of the Journal for the style used in citing references in books and other literature. Full titles of periodicals must be given.

Examples of common references can be found in the ´Style guide for references´.

Use of referencing software. To obtain the style file for this journal, please go to the following websites.

If using ´Reference Manager´, visit http://www.refman.com/support/rmoutputstyles.asp.

If using ´ProCite´, visit http://www.procite.com/support/pcoutputstyles.asp.

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Units
The SI system of units should be used for exact measurements of physical quantities and, where appropriate, elsewhere. The double solidus must not be used in complex groupings of units (i.e. use mg/sheep.day, not mg/sheep/day or mg $sheep^{-1}$ day^{-1}). This Journal uses the abbreviation ´L´ for litre; ´mL´ for millilitre. When using non-standard abbreviations, define the abbreviation where it first occurs in the text.

Spell out numbers lower than 10 unless accompanied by a unit, e.g. 2 mm, 15 mm, two plants, 15 plants, but 2 out of 15 plants. Do not leave a space between a numeral and %, ‰ or ^{o}C.

Mathematical formulae
Formulae should be carefully typed with symbols correctly aligned and adequately spaced. If special symbols must be hand-written, they should be inserted with care and identified by pencilled notes in the margin. Judicious use should be made of the solidus to avoid 2 mathematical expressions wherever possible and especially in the running text. Each long formula should be displayed on a separate line with at least 1 line of space above and below.

Tables
Tables must be numbered with Arabic numerals and each must be accompanied by a title. A headnote containing material relevant to the whole Table should start on a new line.

Tables should be arranged with regard to the dimensions of the Journal columns (8 by 21 cm), and the number of columns in the Table should be kept to a minimum. Excessive subdivision of column headings is undesirable and long headings should be avoided by the use of explanatory notes which should be incorporated into the headnote. The first letter, only, of headings should be capitalised.

The symbol of unit of measurement should be placed in parentheses beneath the column heading. The prefixes for units should be chosen to avoid an excessive number of digits in the body of the Table or scaling factors in the headings. When scaling factors cannot be avoided, the quantity expressed should be preceded by the power of 10 by which the value has been multiplied. For example, the value 0.05 would appear as 5 under the heading 10^{2} x *N* and the value 500 would appear as 5 under the heading 10^{-2} x *N*. Footnotes should be kept to a minimum and be reserved for specific items in the columns.

Horizontal rules should be inserted only above and below column headings and at the foot of the Table. Vertical rules should not be used. Each Table must be referred to in the text, and the preferred position of the Table in the text should be indicated by a note in the margin.

Short tables can frequently be incorporated into the text as a sentence or as a brief untitled tabulation. Only in exceptional circumstances will the presentation of essentially the same data in both a Table and a Figure be permitted: where adequate, the Figure should be used.

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Lettering should be in sans-serif type (**Helvetica or Arial type 1 font**) with the first letter of the first word and proper names capitalised. The x-height after reduction should be 1.2-1.3 mm. Thus for the preferred reductions of graphs to 30, 40 or 50% of linear dimensions, the initial x-height of lettering should be 4, 3 or 2.5 mm respectively. Symbols and grid marks should be the same respective sizes, and curves and axes should then be either 0.8, 0.7 or 0.6 mm thick respectively. Proportionally smaller sizes of type, symbols, grid marks and curve thicknesses should be used for lesser reductions. The following symbols are readily available and should be used:

■ □ ◆ ◇ ● ○ ▲ △ ▼ ▽ ★ ☆ . The symbols + or × should be avoided. Explanations of symbols should be given in the caption to the figure, and lettering of graphs should be kept to a minimum. If information is given in a caption instead of a legend describe the lines and symbols in words (e.g. solid lines, dashed lines, dot-and-dash lines, open circles, solid circles, striped bars, cross-hatched bars and so forth).

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Statistical evaluation of results
Manuscripts must contain a clear and concise description of the experimental design used; with sufficient detail such that, in the case where analysis of variance or regression models are to be used in the statistical evaluation, the reader is quite clear as to how the error term was estimated. The statistical tests should be briefly described and, if necessary, supported by references. Numbers of individuals, mean values and measures of variability should be stated. It should be made clear whether the standard deviation or the standard error has been given.

Nomenclature
The nomenclature of compounds such as amino acids, carbohydrates, lipids, steroids and vitamins should follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature. Other biologically active compounds, such as metabolic inhibitors, plant growth regulators and buffers should be referred to once by their correct chemical name (which is in accordance with IUPAC Rules of Chemical Nomenclature) and then by their most widely accepted common name. For pesticides, the latest issue of ´Pesticides - Synonyms and Chemical Names´ (Australian Government Publishing Service: Canberra) should be followed. Where there is no common name, trade names or letter abbreviations of the chemical may be used. The first letter of a trade name must be capitalised.

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