Investigation into aspects of milk quality and mastitis in sheep

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Associations among udder characteristics, milk yield, milk somatic cell counts, lamb growth and prevalence of mastitis were evaluated in 121 twin-suckled crossbred non-dairy ewes at four, eight and twelve weeks after parturition. Milk production per four hours decreased with time, being 1.09 ± 0.04 litres at week four of lactation, 0.68 ± 0.02 litres at week eight and 0.48 ± 0.01 litres at week twelve, and with respective mean somatic cell counts (SCC) of 0.32 x 10^6 cells mL^{-1}, 0.39 x 10^6 cells mL^{-1} and 0.28 x 10^6 cells mL^{-1}. Incidence of mastitis was 12.7%, 9.8% and 8.9% for weeks 4, 8 and 12, respectively. Of the 20 individuals that displayed SCC indicative of mastitis, only five had elevated SCC at more than one sampling time and none were positive for *Staphylococcus aureus*. However, *S. aureus* was present in 1% of the 474 milk samples analysed but only at weeks 4 and 8. There was inconsistent association between SCC and visual scores for udder depth, udder distention, degree of separation or teat placement ($P>0.05$ for all). Milk volume and weight of lamb was greater ($P<0.05$) for ewes with an udder depth of score 3 compared with score 4 with no other associations evident ($P>0.05$). The incidence of subclinical mastitis was low but present in this flock with the udder characteristics assessed being poor indicators of either mastitis or milk production, and there was no impact on lamb growth.

Keywords: Somatic cell counts; mastitis; udder characteristics; intra-mammary infection

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Chapter 1

Introduction

Intra-mammary infections (IMI) can lead to clinical and sub-clinical diseases in sheep (Gelasakis et al., 2015), with mild to excruciating pain (when the udder is palpated), impacting their productive performance to varying degrees, and potentially affecting the animals' well- being. Pathologically, bacteria and viruses have been widely reported (and occasional instances of fungi or yeast) to cause IMIs. More than 130 bacteria species have been linked with IMI in dairy cows, and a similar number is probable in ewes (Warwick, 2017) as 20 – 30 bacteria species have been widely discovered in the udder of IMI-infected suckler ewes (Marogna et al., 2010; Mørk et al., 2007).

Mastitis is an inflammatory condition of the mammary gland, caused by IMI which impacts the health, welfare and productivity of lactating animals. Numerous studies have described mastitis as the most expensive disease in dairy farming, with economic implication on reduced milk yield and quality, treatment and/or culling of infected animals, and possibly death (Fragkou et al., 2014; Fthenakis and Jones, 1990; Giadinis et al., 2012). Frequently, *Staphylococcus aureus* (causing clinical mastitis) and Coagulase Negative Staphylococcus (sub-clinical mastitis) are commonly diagnosed agents of mastitis in ewes but *Mannheimia spp., Escherichia coli, and Corynebacterium pyogenes*, have also been reported in New Zealand (NZ) (Ekdahl, 1972) and other regions, indicating mastitis as an enzootic disease.

Generally, milk is graded with the concentrations of somatic cells which increase during mastitis infection with identified thresholds that are largely determined based on the impact in milk products. However, less is known about the threshold or impact of mastitis in crossbred sheep where the impact on product quality *per se* is less evident, as milk is consumed by the lamb. Previous investigations into mastitis in crossbred sheep in NZ were over 40 years ago where 0.6-1.7% was discovered in the North Island (Quinlivan, 1968, 1968b) and 0.6-0.7% in the South Island (Clarke, 1972). In the more recent times, Peterson et al. (2017) found the prevalence of clinical mastitis (CM) to be 5% while sub-clinical mastitis (SCM) and its impact on milk and subsequent lamb production have not been sufficiently investigated.

1.1 Objective of the study

- To quantify the prevalence of mastitis in a crossbred ewe flock
- To investigate the effect of mastitis and udder defects on milk quality and lamb production
- To identify the threshold of somatic cell counts (SCC) that may impact milk and lamb production

1.2 Justification

Mastitis-infected sheep are widely hypothesized to show greater SCC and bacteria concentrations, which are expected to impact the ewe's health and productive performance (milk quality and lamb growth). The ewes in the current study has never been evaluated for mastitis, hence, this is a preliminary investigation. Therefore, the results will be compared with previous studies across NZ, Australia, Europe and other parts of the world.

Chapter 2

Literature review

2.1 Mastitis in sheep

Mastitis is an inflammatory condition of the mammary gland, caused by intra-mammary infection (IMI) which usually affects the health status, welfare and productivity of lactating animals. According to Bergonier and Berthelot (2003), mastitis is important from three perspectives, which are economic (mortality of ewes and lambs, reduced milk production, impaired growth rate of lambs and the costs associated with treating infected animals), hygiene (risk of bacterial infections) and legal aspects (regulations on raw milk standards). However, milk from non-dairy ewes, such as crossbred sheep, is rarely considered for consumption due to the low milk production compared with dairy ewes, but a subsequent effect of the disease on lamb performance is possible and not well characterized. Recent studies have been able to shed light on the effects of somatic cell count (SCC) and milk bacteriology with regards to the categories of mastitis i.e., SCM and CM in dairy ewes but the effects in non-dairy ewes have not been extensively investigated in NZ. Further, the aspect of udder characteristics and subsequent effect on lamb growth is not well characterized.

2.2 Somatic cells

Somatic cells are injury- or infection-response cells, mostly comprised of leucocytes (white blood cells) which are of different types; e.g., neutrophils, macrophages, and lymphocytes. These leucocytes act as defense cells for the animal's immune system and they are passed into milk through the blood to combat infectious organisms in the mammary gland. In cases of IMI in sheep, leucocytes are prompted by the macrophages and lymphocytes to eliminate infectious organisms from the udder through inflammatory processes (Menzies et al., 2013) which may result in swelling, redness and secretion of sanguineous liquid in the gland. According to Menzies et al. (2013), the quantity and type of somatic cells (leucocytes) in milk vary with the magnitude of udder infections. This was further described with a linear scoring system which portrayed SCC in relation to the incidence of mastitis (Table 2.1). Furthermore, milk from healthy dams has been widely reported to contain low SCC that may increase or

fluctuate when an infection is present. Romeo et al. (1996) discovered 185 × 10^3 mL^{-1} SCC in the udders of ewes that were free of IMIs during lactation, whereas 1445 × 10^3 mL^{-1} SCC was linked with IMI-infected ewes in the same study. Similarly, Bergonier et al. (2003) reported a corresponding threshold of 2-3% epithelial cells, 10-35% polymorphonuclear neutrophil leukocytes (PMNL), 45-85% macrophages and 10 -17% lymphocytes in IMI-free lactating ewes. However, IMI-infected ewes showed greater SCC with the influx of over 90% neutrophils (Harmon, 1994; Miller and Paape, 1985) in the milk, suggesting a relative change in SCC due to the magnitude of infection.

Table 2.1: The different types and proportions of somatic cells in milked uninfected sheep milk (Menzies et al., 2013)

Cells	Quantity (% per milk volume)	Functions
Macrophages	80	Alarms and signals the udder for infectious pathogens.
Lymphocytes	15	
Polymorphonuclear leucocytes	< 5	Becomes abundant in milk based on the magnitude of infection and destroys pathogens in the udder.
Epithelial duct cells	<5	Lines the walls of the alveoli to produce milk nutrient components in the udder

Raw milk is generally graded with SCC thresholds which are associated with the health status of udder halves in lactating animals. Some studies have reported that ewes with SCC lower than 0.5 × 10^6 cells mL^{-1} indicate a healthy mammary gland while SCC greater than 1.0 × 10^6 mL^{-1} has the tendencies of either CM or SCM, and SCC between 0.5 × 10^6 mL^{-1} and 1.0 × 10^6 mL^{-1} is an indication of suspected IMIs which could be verified with bacteriological examination of the milk samples (Berthelot et al., 2006). Although, these thresholds have been established in dairy ewes with varying values, but compelling SCC are also probable in non- dairy ewes.

2.3 Risk factors and causes of mastitis

IMIs are mostly caused by predisposing factors, which emanate from farm-level practices. Minimizing contact between infected and non-infected ewes to ensure a disease-free flock is

unlikely to be practical on extensive farms. Mastitis can be reduced if the following predisposing factors are considered as routine management practices.

2.3.1 Aetiological agents

Worldwide estimates suggest *Staphylococcus aureus* as the major cause of mastitis in lactating animals, but other bacteria species, staphylococcus strains, and viruses, may cause mastitis in sheep to varying degrees. CM is associated with clinical signs (of the infection when examined) without further laboratory tests (e.g., SCC and or bacteria analysis). In sheep, CM is most often linked with *Staphylococcus aureus* and *Mannheimia haemolytica*, resulting in lesions, inflammation or gangrene around the udder (Barber, 2016a). *M. haemolytica* has also been found in the nasopharynx of lambs and may subsequently cause IMIs in ewes through cross-suckling (Barber, 2016a; Scott and Jones, 1998). Coagulase-negative staphylococci, *Streptococcus spp.* and enterobacteria have also been cultured from the udder of lactating ewes (Arsenault et al., 2008; Bergonier et al., 2003; Lafi et al., 1998; Mørk et al., 2007).

Koop et al. (2010) described CM to be influenced by several factors that may include dystocia, breed, region and the number of lambs born. For example, an increased risk of CM was reported in ewes with heritability ratio estimate of 3.1 compared with the single- or twin-bearing ewes (Arsenault et al., 2008) thus indicating the potential effect of higher suckling frequency which could result in lesions or bruises and subsequently CM as related in many sty studies. Generally, the incidence of CM in ewe flocks is usually below 5% per year (Bergonier et al., 2003; Contreras et al., 2007), displaying SCC of more than 1,000,000 cells/mL and the presence of pathogenic bacteria in milk (Świderek et al., 2016). However, SCM appears difficult to detect (compared with CM) unless determined by SCC in the milk. Giadinis et al. (2012) described SCM as the primary cause of the common “milk-drop syndrome” in lactating ewes i.e. reduced milk yield and with no clinical signs. Similarly, reduced milk yield may cause suboptimal growth in lambs (Fthenakis and Jones, 1990). This is a resultant effect of the low milk production which could be insufficient for the lambs. Furthermore, coagulate negative staphylococcus bacteria has been widely reported in cases of SCM. *Staphylococcus hyicus, Staphylococcus intermedius* and *Staphylococcus schleiferi,* i.e., coagulase-positive bacteria species have also been linked with SCM (Gelasakis et al., 2015) along with other probable bacteria species. Various studies have suggested that incidences of SCM in dairy ewes will

display about 200,000 - 1,500,000 cells/mL SCC (Barber, 2016b) but this may differ in non-dairy ewes.

2.3.2 Teat and udder damage

Menzies and Ramanoon (2001) hypothesized the occurrence of IMIs in non-dairy ewes where heavy lambs caused lesions/bruises through teat-biting and rough suckling, thereby exposing the udder to bacterial invasion, e.g., Pasteurella spp. (Marsh, 1932; Tunnicliff, 1949). Less has been reported on the relationship between udder or teat lesions and the risk of mastitis but (Watkins et al., 1991) found insignificant association and characterized the effect as anecdotal, suggesting further investigations. However, Larsgard and Vaabenoe (1993) observed a significant relationship between the number of suckling lambs and IMI with an annual mastitis incidence of 6.8% in some Norwegian non-dairy breeds. It was further suggested that the infection occurred due to vigorous suckling which predisposed the udder to the infection. This could also occur when an ewe is raising more than two lambs, culminating in frequent and vigorous suckling events which may result in udder lesions.

2.3.3 Suckling and cross-sucking of lambs

Orphaned or extra lambs tend to occur in sheep flocks at lambing times due to reasons which may include IMI (leading to insufficient milk for the lambs and limited sucking access if the disease is of high severity), death of the ewe after birth, and early separation/weaning (as mostly practised in dairy production). The mammary gland may also be inaccessible to weaker lambs i.e. the extra lamb due to high litter size. This often occurs in flocks with ewes that have high incidence of triplets and quadruplets (Thompson et al., 1993). Waage and Vatn (2008) reported higher chances of developing IMIs through frequent teat bites and competitive sucking in ewes that produced at least two lambs. (Koop et al., 2010) also discovered increased risk of CM in ewes suckling multiple lambs.

Suckling lambs contribute to the transmission of *M. haemolytica* through the teat duct of ewes (Fragkou et al., 2011) thereby causing mastitis or IMIs. Many studies have reported the presence of *M. haemolytica* in the respiratory tract of lambs (at the nasopharynx region) and suggested a potential transmission to the dams during suckling events. Fragkou et al. (2011); Gougoulis et al. (2008) also discovered isolates of the bacteria in the udder after a suckling event and suggested that the transmission was through the teats. Furthermore, Barber

(2016a) described a subsequent spread of *M. haemolytica* during cross-sucking of lambs in some ewe flocks, which later resulted in IMI. This suggests that the ewes used to cross-suckle or foster lambs are at great risk of developing IMIs which may subsequently be transmitted across flocks.

2.3.4 Age

The age of ewes may determine the period of predisposition to certain diseases. The prevalence of IMI has been widely described to escalate in older ewes, and with increased odds of reoccurrence overtime. Al-Majali and Jawabreh (2003) discovered a significant relationship between mastitis and age in ewes, with 18.3% SCM prevalence ratio which increased with age and a greater impact in multiparous ewes than in the primiparous ewes (Indrebø, 1991). Similarly, Waage and Vatn (2008) found increasing odds of CM (9.24 times) reoccurrence in 5-year-old ewes compared with the 1-year olds. This also manifested in ewes with multiple lambs compared with uniparous ewes, suggesting that a previous IMI-infected ewe can be re-infected overtime. However, Quinlivan (1968) did not find any relationship between age and incidence of mastitis in many Romney flocks which was made up of 19,427 ewes but did report a greater susceptibility to CM which was associated with lesions/bruises as a result of frequent suckling of lambs. This result may be because the flocks were located across different environment. In a more recent study by Peterson et al. (2017), with a smaller sample size (1824 ewes), discovered a correlation between age and mastitis, and a higher incidence of mastitis in older ewes. This may be because the older ewes are more likely to suffer from other health issues which may further increase their susceptibility to mastitis.

Furthermore, many studies have reported a greater IMI-susceptability in older ewes/multiparous ewes, and generally having higher SCC than primiparous ewes. Fthenakis (1994) found high SCC values (1.0×10^6 mL^{-1}) in lactating ewes (8.9%), which were more predominant in older/multiparous ewes (2.9 – 19.4%) than in the young/primiparous ewes (0 – 7.4%). Conversely, Green et al. (2016) identified a greater potential for mastitis in first-time lambers, possibly because the skin of the teat is quite tender (compared with older animals) while the mammary gland is still developing. It was also described that younger ewes may take longer to appropriately suckle their lambs because of no prior experience, thereby increasing the risk of teat lesions and udder damage via suckling adaptability. These findings are yet to be fully validated, thus, they require further investigations.

2.3.5 Dry period

Dry period is the period between two lactation or lambing seasons. This period has been described in many reports to permit involution of the mammary gland and restore the lining of the udder before lactation starts again. According to Green et al. (2016), the dry period can be a risk factor for animals (e.g. dairy cows) to acquire IMIs in the following lactation because udder quarters that get infected during the dry period are more likely to go on to develop mastitis. This was described in association with dry cow therapy (DCT) which was mostly common in dairy cows. DCT is part of the five-point plan that was developed to treat or prevent IMI in the 1960s. This therapy involved the use of a long-acting antibiotic, placed into the mammary gland through the teat to remove pre-existing infections and prevent new ones during the dry period. Teat sealant is also used, as the long-acting antibiotic may be insufficient to protect the udder over the whole dry period. However, in the more recent times, the dairy industry is moving away from the conventional methods of treating IMIs in cows because studies have shown that the use of the five-point plan and DCT does not reduce the incidence of mastitis rather, it changes the type of bacteria that causes mastitis (Green et al., 2016).

While in sheep, dry period antimicrobial therapy has been established in dairy ewes with evidences of improved cure rates and decreased level of new infections but varied across different studies (Menzies and Ramanoon, 2001). However, Green et al. (2016) suggested that DCT is unlikely to become a standard practice in sheep due to cost and the recent clamour to minimize antibiotics usage in animals as regards public health aspects. Similarly, long-acting antibiotics may not be as effective for sheep because the dry period is longer than in cows. Although some studies discovered that administering parenteral antibiotics to ewes over dry period may reduce incidences of CM in dairy ewes (Barber, 2016a; Quinlivan, 1968) but these effects are yet to be validated in non-dairy ewes.

2.3.6 Nutrition

Animals tend to cure themselves from pathogens/disease-causing organisms by biological body processes i.e. immunity, to destroy the pathogens and ensure normal body function. According to Green et al. (2016), it is important to provide ewes with adequate feed (for energy and protein) during pregnancy and lactation so that they can produce healthy lambs. This is due to the increased physiological stress during these periods, whilst experiencing increased energy demands. Underfeeding or deficiencies of adequate nutrient requirement

have been widely reported to predispose ewes to CM and other IMIs, causing low milk production which subsequently lead to lesions caused by hungry suckling lambs.

Increased risk of CM and SCM in ewes with deficient in vitamin A and selenium has been reported, causing reduced integrity and functionality of the epithelial defences in affected glands (Giadinis et al., 2011; Koutsoumpas et al., 2013), hence, well-nourished ewes may be better able to fight IMIs. Similarly, Onnasch et al. (2002) suggested that mastitis-resistance could be influenced by nutrition, and poor body condition score (BCS) may increase the risk of IMIs. It has also been suggested that maintaining ewes at BCS at 3+ through adjustments of feed rations to include more protein at parturition and subsequently in lactation (Green et al., 2016) will increase production performance.

2.3.7 Ewe body condition score (BCS)

Generally, the periods of pregnancy and lactation in ewes are associated with extra energy demands that must be compensated by an adequate feeding regime. During this period, ewes may mobilize their energy reserves (i.e. fat, which is mostly present around the backbone near the ribs region, lumbar processes and top of the pelvis) to support body requirement, hence, a potential loss in BCS. Ewes in poor body condition at lambing (below BCS 3) or with inadequate nutrition, may not produce enough milk, causing hungry lambs to frequently bunt the udder and perhaps bite teats in attempts to draw more milk (Green et al., 2016), thereby predisposing the ewe to IMIs. Menzies et al. (2013) hypothesized that ewes at BCS 3.0 - 3.5 will usually mobilize back fat during lactation (to about BCS 2.5 or lower), indicating that body energy reserves in ewes are mobilized for lamb production. Similarly, Mathias-Davis et al. (2011) demonstrated that ewes with lower BCS (3.0 - 3.5) at weaning showed better lamb production compared with higher BCS ewes (3.5), suggesting that ewes should be provided with high-quality nutrition for persistent lactation, production of healthy lambs and recovery from the lost BCS (due to lactation). However, many studies have established that ewes are highly predisposed to disease infection with inadequate nutrition, causing low BCS and affecting their immunity (Green et al., 2016). Similarly, ewes at low body condition (lower than BCS 3) are highly predisposed to SCM and CM (Barber, 2015), which may further cause suboptimal lamb growth.

2.3.8 Environmental conditions

The environment where animals are kept could influence the type of disease-infections (e.g. IMI) and the causative organisms. This may result from infection of the udder by microbes that come from the environment. The sources of microbes that causes environmental mastitis may include; manure, bedding, feed, dust and dirt, mud, water and contaminated equipment. The term "environmental mastitis" was used in a study by Sharma et al. (2011) to describe environmental pathogens (such as; *S. dysgalactiae, S. uberis, Corynebacterium bovis* and Coagulase negative Staphylococcus) which caused SCM with increases in SCC (> 200,000 cells/ml). Although, this study was based on cows, similar pathogens have been widely reported in ewes. Particularly, a 10-year survey by Gelasakis et al. (2015) revealed isolates of various bacteria in ewes' milk, causing SCM and CM. These include; *coagulase-negative staphylococcus* and *Staphylococcus aureus*, being responsible for SCM and CM respectively in dairy ewes (Bergonier et al., 2003; Contreras et al., 2003; Contreras et al., 2007; Mørk et al., 2007). While *Mannheimia haemolytica* or *S. aureus* were mostly found in most CM cases in non-dairy ewes.

2.3.9 Hygiene Practices

Generally, good health status in ewes will support efficient control of IMIs by preserving an effective immune system. Frequent cleaning and disinfection of the ewes' shed, and maintenance of appropriate stocking densities with a relative land-space of 7 m^3 per animal have been reported to reduce IMIs and incidence of SCM caused by environmental pathogens (Sevi et al., 2001). This is largely associated with animals in intensive production systems (e.g. dairy ewes) where the animals are usually checked for infections or any other abnormalities during the daily milking unlike the non-dairy ewes which are rarely checked. Table 2.2 shows some disease management practices that could ensure a healthy sheep flock.

2.3.10 Breed susceptibility

Although mastitis resistance could have a genetic base, there are no reports to evidently show that a particular sheep breed is IMI-resistant. Bonnefont et al. (2011) described genetic selection through quantitative trait loci of DNA parameters, which correlates with variation in sheep phenotypic characteristics (e.g. udder trait) as a potential basis to raise an IMI–free flock. Furthermore, Barillet et al. (2001) researched breeding programmes in dairy sheep, using indirect selector traits i.e. occurrence of CM or SM and SCC values to breed new genetic

lines. There was a low incidence of SCM (10%) and CM was 5% with heritability estimate of 0.15 based on SCC. Rupp et al. (2009) also reported low incidence of IMI and a corresponding low SCC in some Lacaune dairy ewes, using the frequency and duration of mastitis in the parent lines. These results may be different in non-dairy breeds because IMIs are predominantly reported in dairy ewes compared with their non-dairy counterparts due to machine-milking effects (Albenzio et al., 2003; Contreras et al., 2007). However, the history of mastitis in flocks may give insights about the heritability of mastitis resistance in ewes. Larsgard and Vaabenoe (1993) suggested resistance to mastitis existed in ewes that were never diagnosed with the disease throughout their production cycle. Similarly, breeding for resistance against mastitis with indirect predictor trait (e.g. SCC) was used in some French Lacaune sheep for improved resistance. Rupp et al. (2009) also reported that using lower SCC values for selection purposes in animals could be linked to subsequent reduction of incidence of IMIs. If these investigations persist (and are replicated in non-dairy ewes), such ewescould be a valuable genetic resource to breed animals with a low incidence of mastitis. In addition, good udder traits and management practices could aid the selection of ewes with low incidence of IMI for optimum production.

Table 2.2: Disease management practices for sheep flocks (Garforth et al., 2013)

Practice	Description
Avoid new diseases being brought onto the farm by introduction of infected animals	- Provision of isolation unit for sick and new/replacement stock before joining with the remaining farm stock - Ensure sourcing animals from credible suppliers with good production/health history of new stock - Proper fencing to limit wildlife contact with animals
Avoid disease being brought onto farm by visitors and neighbouring farms	- Prevent visitors from touching animals without your knowledge - Avoiding grazing animals on common land
Avoid spread/multiplication of disease on the farm	- Taking specific action to keep animals clean (high standard hygiene) - Neonatal disease control: hygiene in lambing areas/separation/cleansing/disinfection/afterbirth - Disposal or rearing infected lambs separately from each other

Culling is usually adopted management practice to avoid the presence of ewes with abnormal udder and persistent disease condition. Gelasakis et al. (2015) suggested that culling of ewes at the end of a lactation to reduce incidence risk of mastitis in a flock but replacement ewes may be too expensive from the economic aspect. Conversely, Mavrogianni et al. (2011)

advocated the following criteria for culling: (i) ewes with permanent damage in at least one-half udder, (ii) animals with chronic infection (iii) animals with incidences of reoccurring mastitis (iv) animals with no full response to mastitis treatment during the previous lactation. These criteria may reduce the cost of controlling the disease, reduce sources of potential infection and decrease incidence of IMIs. Currently in NZ non-dairy ewes, culling due to udder defects is common at weaning (Griffiths, 2016), although there is no data to support the rationale for this approach and whether udder defects may naturally resolve during the dry period.

2.4 Diagnosis of mastitis

As described by Fragkou et al. (2014), mastitis can be diagnosed with clinical examination, bacteriological tests, cytological examination of milk by using fluor-optoelectronic counters and microscopic cell counting), and indirectly by using electrical conductivity, imaging techniques (ultrasonography, endoscopy, infrared thermography), California mastitis test (CMT) and Whiteside test (WST) (Fragkou et al., 2007; Fthenakis, 1995) as discussed below.

2.4.1 California mastitis test and Whiteside test

CMT and Whiteside test (WST) are indirect methods of detecting the increased number of SCC or leucocytes in the milk of lactating animals because they do not give actual SCC (Menzies and Ramanoon, 2001) but an assumed estimate. CMT is based on the principle of rupturing the leucocytes in the milk and releasing their deoxyribonucleic acid (Fthenakis, 1995) and estimating the SCC with a scoring system. On the other hand, the WST shows a precipitate of absorbed serum solids and fat globules from the nucleic acids of the leucocytes which forms a sodium salt with sodium hydroxide (Schalm et al., 1971). These methods are rapid and easy to carry out but require further laboratory analyses i.e. bacteriology and SCC with fluor-optoelectronic counters (Paape et al., 2001) or direct cell counters.

2.4.2 Clinical examination

Clinical examination involves assessment of the udder and teats for visible anomalies. The udder is usually assessed for lumps, abscesses, lesions, and structural conformation (which may deviate from normal IMI-free udder) through palpation and physical examination (Mavrogianni et al., 2005). Further diagnostic tests may involve bacteriological and cytological evaluations on milk or sanguineous liquid drawn from the teats (Gelasakis et al., 2015).

However, recent discoveries have shown varying udder infrared temperatures in sheep with mastitis presumably indicating the presence of inflammation e.g. 36.3^{o}C - SCM and 35.89^{o}C - CM as mean temperatures in cases of mastitis (Martins et al., 2013). This may well become a significant indicator in the examination of IMIs.

2.4.3 Infrared thermography (IRT)

IRT is an imaging technique which is non-invasive. Berry et al. (2003) investigated the incidence of mastitis in sheep by using IRT. 95% cases of SCM had higher udder temperatures compared with the normal sheep core temperature threshold (38.3^{o}C – 39.9^{o}C) but the absence of clinical signs made it difficult to determine the extent of the infection. Similarly, Martins et al. (2013) established varying high temperatures and SCC values in ewes showing incidences of mastitis e.g. 39.02^{o}C, between 250,000 and 500,000 cells/mL (SCM group) and 38.4^{o}C with above 500,000 cells/mL (CM group). This was further explained in Figure 2.1 where mastitis was discovered in both udders in an ewe and in a single udder in another ewe.

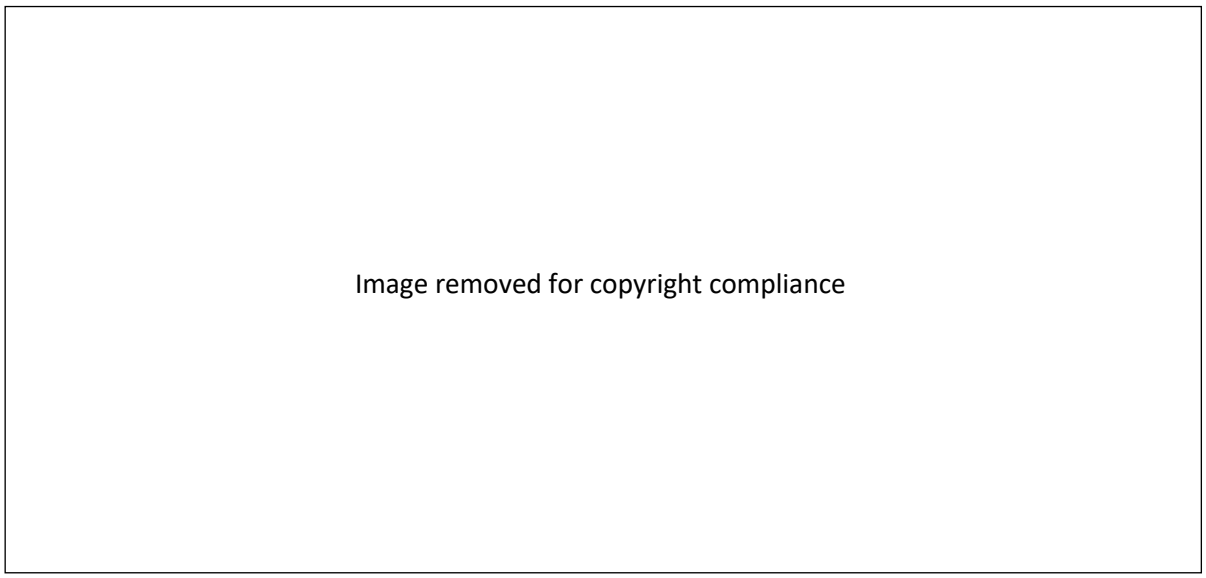

Figure 2.1: Thermographic images of the udder of ewes with mastitis at both sides (left) and an udder with mastitis at one side (right) (Martins et al., 2013)

2.4.4 Ultrasonographic examination

Fragkou et al. (2014) described ultrasonography as a quick tool for detecting and monitoring structural changes in the teats and mammary glands with non-invasive and painless processes. This method can evaluate the entire udder by moving a probe along the gland surfaces to

detect lesions within the teat and the mammary parenchyma (Mavrogianni et al., 2004), complementing the clinical examination. Similarly, computer tomography scanning (CT - Scan) is another technique that is performed but a clear distinction is that CT - scan can produce cross-sectional images with x - rays which can be viewed slice by slice whereas ultrasound uses high frequency sound waves to produce pictures of the body organ. In general, there are less reports on the viability of ultrasonographic methods of examination thus, require further investigation.

2.4.5 Endoscopy

Endoscopy is an inversion process which involves insertion of an endoscope into the teat orifice or the mammary parenchyma (Kiossis et al., 2009, 2012) to evaluate the intra-mammary defects. This gives a real-time image of the activities in the udder but there are concerns of increased risk of mastitis by introducing bacterial flora into the udder from the teat duct (Fragkou et al., 2014; Fragkou et al., 2007) while using the endoscope. However, this process requires expensive equipment and experienced personnel which renders it unpopular in evaluating IMIs.

2.4.6 Electrical conductivity

Electrical conductivity in milk is based on the concentration of ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-) at early stages of mastitis, detecting lesions in the mammary epithelial cells caused by pathogenic activities. Milk electro-conductivity is measured at each mammary gland as milk flows through milk tubes with conductometers (Romero et al., 2012). Díaz et al. (2012) described inter- and intra-animal variations in the milk conductivity values which are dependent on daily change and gland variations, hence, there is a need to calibrate with specific algorithms to detect mastitis condition. This method can be used at the farm level with appreciable accuracy. However, it is generally designed for cows (with 4 teats) but there are studies regarding potential application in ewes (Hamann and Zecconi, 1998; McDougall et al., 2001).

2.4.7 Bacteriological examination

Fragkou et al. (2014) described bacteriological examination as the gold standard for aetiological diagnosis of diseases. Bacteria isolates of more than 10 Colony Forming Unit/mL of milk from the milk sample of a mastitis-infected ewe is sufficient to demonstrate the

aetiological role of disease-causing organisms (Contreras et al., 2007; Jones and Lanyon, 1987). However, repeated sampling and bacterial examination is advised for efficacy. On the premise that the IMI is suspected to be a systemic disease rather than only in the milk, immunological examinations need to be performed on blood and milk samples based on the actual disease to be investigated e.g., *Mycoplasma spp.* infection (Nicholas, 2002; Nicholas et al., 2008) or *Staphylococcus spp.* infection (Cuccuru et al., 2011). Further, immunoassays can be used in milk to detect antigen or antibodies of microorganisms in milk which may be indicators of IMIs.

2.4.8 Cytological examination

Measurement of the number of leucocytes in milk i.e. SCC is considered the best method for investigating inflammatory response of ewes with mastitis. SCC are directly measured by fluor-optoelectronic counters or microscopic cell counting in the lab, which enumerates the particles with DNA in milk (Contreras et al., 2007; Gelasakis et al., 2015; Paape et al., 2001; Paape et al., 2007) while indirect measurement can be done using either of California Mastitis test or Whiteside test (Fragkou et al., 2014) which are sheep-side estimates for SCC in the field. Some studies suspected inaccuracy of SCC instrument with milk samples that are frozen/stored for too long (e.g. 60 days). This was demonstrated in a study by Martins et al. (2013) where a lower SCC (234,000 cells/mL) was discovered in milk samples stored for over 60 days at - 20^{o}C. However, Zeng et al. (1999) found that milk samples stored in ice (not frozen) for up to 3 days (without preservative) did not affect the SCC. The addition of bronopol and refrigeration of the milk samples can however increase the storage time to about 25 days (Sánchez et al., 2005).

2.5 Incidence of mastitis

The incidence of mastitis has been widely reported. According to Green et al. (2016), SCM could affect more than 50% in a flock due to the contagious and transmissible nature of mastitis. There is also a possibility of higher SCM prevalence because it does not show any clinical sign unless with pen-side or laboratory evaluation. Furthermore, numerous studies have described the impacts of SCM on milk production which may subsequently affect lamb growth as a result of inadequate production. Giadinis et al. (2012) established that “milk-drop syndrome” i.e. low milk production is associated with SCM in sheep flock based on the following criteria (i) when there is over 30% reduction in the total flock milk production (ii) when over 25% of the ewes are affected and, (iii) when individual ewe has over 25% reduction

in milk yield. The inadequate milk production was suggested to cause sub-optimal lamb growth rate in mutton-type production flocks (Fthenakis and Jones, 1990).

On the other hand, CM depicts visible clinical signs (abscesses, gangrene, lumps, etc.) as shown in Figure 2.2, with pain when the udder is examined (Fragkou et al., 2014), and this sometimes led to sanguineous udder secretions which may not contain adequate nutrients for lamb growth. However, the effect of CM varies across flocks at about 1% - 5%, Peterson et al. (2017) also discovered 5% CM cases in some NZ flocks, and with 0.6% of the same flock displayed different udder defects which may negatively impact milk production. Some studies have suggested that CM can be managed if detected early, but a flock is usually at increased risk of mastitis infection due to its contagious and transmissible nature (Barber, 2016a).

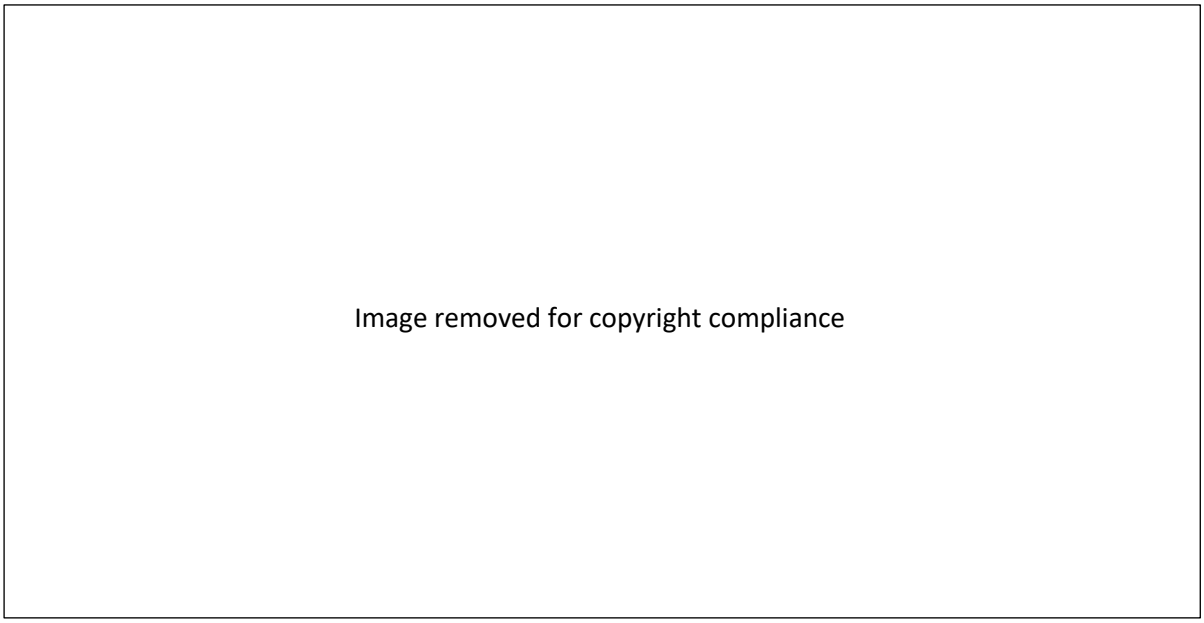

Figure 2.2: A lactating ewe having clinical mastitis with lumps and swollen udder (Green et al., 2016)

2.6 Control of mastitis

According to many reports, injectable antibiotics and anti-inflammatory drugs are used to treat mastitis in ewes but the high treatment cost per animal is usually compared with the culling value and the expected recovery period (Bergonier et al., 2003) which determines the efficacy of treatment. The knowledge of mastitis causative agents and possible transmission

routes have been improved overtime but there is still no effective control strategy in place because mastitis is a multi-factorial disease and there is a high potential of reoccurrence in previously infected animals (Green et al., 2016). The clinical signs of IMIs have been widely described to be easily treated unlike the bacteriological impacts which still linger (i.e. residual effect) even after treatments (Abu-Samra et al., 1988; Fernández et al., 1999; Lerondelle and Ouzrout, 1990; Podstatzky-Lichtenstein et al., 1998; Poutrel et al., 1997), and may subsequently cause decreased milk production and recrudescence of clinical IMIs.

Gelasakis et al. (2015) suggested that antibiotic-therapy should be accompanied with careful consideration based on the appropriate withdrawal periods for milk from animals under medication. This is from public health concerns since antibiotic residue in animal products (especially milk and meat) have been found to cause allergy in sensitive consumers (Chand et al., 2000) thereby reducing milk quality. However, the milk from non-dairy ewes is usually not consumed unlike the one from dairy ewes and there no reports on the impact of such milk in suckling lambs. Vaccine availability for mastitis is still in its infancy (Green et al., 2016) hence, focus on management practices i.e. hygiene, nutrition and prevention of spread, will always be critical to reduce incidence of IMIs. Further, immediate treatment of mastitis is required to prevent damage to udder function and to minimizes sources of infection to the rest of the flock.

2.7 Milk production in sheep

Generally, milk yield and length of lactation in sheep vary across breeds (i.e. dairy and non-dairy breeds). The East Friesian breed is widely reported as the highest milk producer with around 3100 g/day (at peak lactation) and 500 – 700 kg total milk yield and having the longest lactation length (around 240 days) compared with non-dairy breeds (90 - 150 days) (Green et al., 2016; Sheep, 2017). According to (Menzies et al., 2013), the total milk production in sheep is dependent on the shape of the lactation curve, which deals with the time and height of peak milk production (maximum daily milk yield during lactation) and the length of lactation. However, the length of lactation and peak milk production are influenced by breed, photoperiod (daylight length), nutrition, lactation number (first- or second- time lactation), stress and pain at milking, milking frequency and presence of IMIs (Menzies et al., 2013; Pollott and Gootwine, 2004). Some studies have demonstrated that milk production is associated with litter size i.e., in twin- and triplet- bearing ewes, thereby producing about 20 litres more milk per lactation and 1% increase in lactation persistency than in single-bearing ewes. This

was recorded in some Assaf dairy breed in Israel where the animals were kept under intensive management system and surprisingly, the lambs were weaned at birth (and reared artificially) on the premise of accurate measurement of the ewes' milk production (Pollott and Gootwine, 2004). Similar effect is possible in non-dairy breeds, but some differences may occur because they produce lower quantity of milk (averagely 47 – 103 litres) compared with the dairy breeds which produces about 234 – 354 litres of milk per lactation (Shrestha et al., 2008). Nieto et al. (2018) reported a 30% reduction in milk yield of merino ewes bearing single lambs compared with the twin-bearing ewes, and there was no effect of production in the dams suckling ewe lambs or ram lambs. This impact of milk production was further explained as seen in Figure 2.3 where there was a consistently higher milk production in twin-bearing ewes than the single-bearing ones, and with a 33% and 28% decline from days 28 and 56 for the single- and twin-bearing ewes respectively. While the sharp decline from day 56 to 70 (57% for the singles and 42% for the twins) was associated with lambs' decreasing dependence on milk. However, the milk yield between parturition and day- 28 was not given in the study, this may be in order not to compromise the growth and development of the lambs hence, the ewes were milked near their peak lactation period (Bencini et al., 1992a; Bencini and Purvis, 1990).

In addition, multiparous ewes have higher peak milk production and lactation persistency than the primiparous ewes. It was further observed in some studies (Bencini and Pulina, 1997; Paten et al., 2017; Snowder and Glimp, 1991) that heavier ewes (multiparous ewes) produced more milk than their lighter counterparts i.e. primiparous ewes. This may be because the multiparous ewes are usually older and matured than the primiparous which are still undergoing physiological developments.

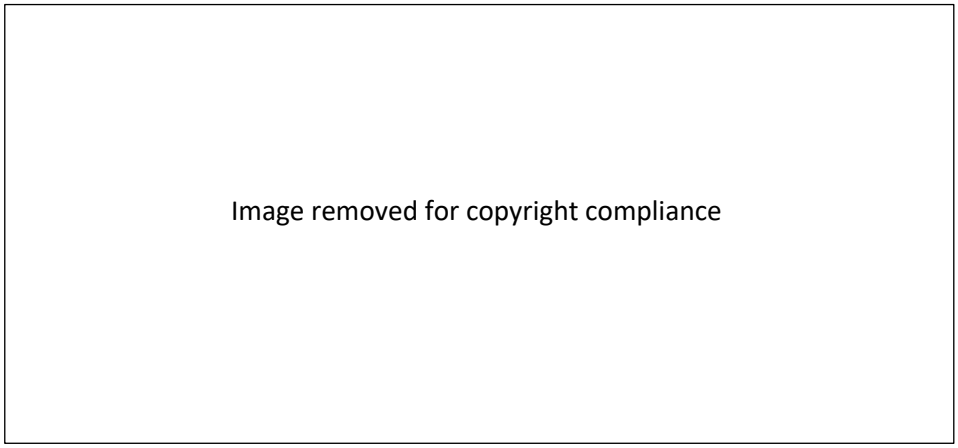

Figure 2.3: Lactation curve of single- vs twin- bearing multiparous non-dairy ewes (Snowder and Glimp, 1991)

2.7.1 Milk Composition in Sheep

The nutrient composition of sheep milk varies across breeds. Many reports have established that sheep milk contains higher fat and protein content compared with cow milk (see table 2.3). This was observed in some non-dairy sheep breeds in Pakistan, where the total solid, fat, protein, and reduced lactose (18.53%, 8.96%, 6.57%, and 3.57% respectively) compared with cows and goats (Kanwal et al., 2004). Similarly, higher protein and fat contents (at 1.2- and 1.5 - fold) were recorded in some dairy Italian ewes compared with dairy cow and goats (Pulina and Bencini, 2004). This may be due to the uniqueness of sheep and other complex biological references. However, an earlier study (Geenty, 1979) found no difference in the milk composition of crossbred ewes (which comprised of Dorset, Romney, Corriendale and Dorset X Romney crossed) but recent NZ studies discovered higher variation in the nutrient composition of Romney and Merino ewes (fat 8.24%, protein 5.64%, lactose 6.06% and total solid 14.48%) compared with non-dairy cow milk (Table 2.3). These variations may be influenced by age of the ewes, stage of lactation, nutrition, climatic conditions, management practices, and presence of disease infection, thereby prompting further investigation.

Table 1.3: Nutrient composition in sheep milk vs cow milk – Reproduced from different studies (Nieto et al., 2018; Paten et al., 2017; Rodrigues et al., 2014)

	Non-dairy Sheep milk		Non-dairy Cow milk	
Components	**Romney**	**Merino**	**Angus**	**Angus crosses**
Total protein (%)	5.64	4.12	2.90	3.08
Fat (%)	8.24	4.10	3.21	3.71
Lactose (%)	6.06	5.49	4.65	4.72
Total solids (%)	____	14.48	11.58	12.42

2.7.2 Factors that influence milk production in sheep

As a rule of thumb, the overall aim of most modern sheep husbandry is to achieve optimum productivity. The dairy farmers consider high-quality milk and increased milk yield while the farmers of non-dairy sheep are usually particular about fecundity i.e. production of more lambs to increase flock size thereby increasing meat and or wool production, but less attention is paid to the milk yield and quality, which may be under produced for optimum lamb

production. Numerous studies have attributed many factors that may affect the rate of milk production in ewe flocks, thereby influencing the selection criteria by sheep farmers to ensure optimum production.

Milk production in ewes usually commences from an ascending phase (early lactation) to the highest i.e. peak lactation, followed by a decreasing phase (late lactation) until drying-off. Peak lactation in ewes vary across different breeds (with different ewe ages and diverse genotypes). For example, some NZ Romney ewes produced the greatest milk yield around day-10 of lactation (Paten et al., 2017) while some East Friesian crosses peaked around the first week of lambing (Peterson et al., 2005). This is followed by decreasing levels of production until around 200 days in dairy ewes (Pulina and Bencini, 2004) and 90 days in non-dairy ewes (Menzies et al., 2013). Many studies have associated adequate nutrition to influence milk production and promote the length of lactation. This was described as one of the most important sources of variation in ewes' milk yield and nutrient content throughout lactation (Sevi et al., 2004). Similarly, Menzies et al. (2013) associated nutrition as one of the factors that influence the level of peak lactation and lactation persistency in dairy ewes. This effect may be different in non-dairy ewes, but it could give insights about the available milk for lamb growth because lactation period is associated with the highest nutrient requirement in the ewe's annual production cycle (Snowder and Glimp, 1991). However, Peart (1982) suggested that inadequate nutrition may have minimal impact on milk production but may cause appreciable loss of body weight and energy reserves (e.g. BCS) which may predispose ewes to health risks due to the high-nutrient demand of lactation.

Studies have established the influence of the number of suckling lambs, breed, and stage of lactation in ewe milk production. According to Snowder and Glimp (1991), the number of suckling lambs i.e. litter-size clearly influenced milk production as seen in Figure 2.3 where there was a consistently higher milk production in twin-bearing ewes than the single-bearing ones, and with a 33% and 28% decline from days 28 and 56 for the single- and twin- bearing ewes respectively. This was further demonstrated with around 15% increased milk volume in some NZ Romney ewes which reared twin lambs compared with those that reared single lambs. A similar result was also found in another study where the Suffolk single lambs were found to grow faster (at 8 weeks) with higher weight gain (15.5 kg) compared with the twins (10.7 kg) because the single lambs probably had ad-libitum consumption (Slen et al., 1963) and access to both udder halves compared with the twins that naturally suckle by competitive

advantage. Further, Snowder and Glimp (1991) demonstrated that twin-bearing ewes produced more milk than the singles at every stage of milk production with about 23-58% more from 28 to 56 days post lambing (see Figure 2.3). It was also reported that ewes suckling twins produced 71-149% more milk at late lactation (70 to 98 days) i.e. high lactation persistency. However, an earlier study described greater milk production in twin than the single-suckling ewes but only at early lactation (14 - 42 days) and with little or no effect on lactation persistency at late lactation (Gardner and Hogue, 1964; Treacher, 1983). This effect may be due to environmental circumstances or inherent differences between the sampled ewes or different methods of assessing milk production.

2.7.3 Udder conformation

Animal selection criteria by dairy sheep farmers is usually based on milk production (quantity and composition), whereas the appraisal of udder morphology is less taken into account for optimum production (Gelasakis et al., 2012). Labussière (1988) considered udder conformation as a major functional trait for the evaluation of ewe milkability. Perhaps, this may also manifest in non-dairy ewes and could determine the quantity of available milk for suckling lambs. Further, defective udder conformation could increase the chances of IMI, which may reduce the quality and quantity of milk during lactation.

Many studies have linked poor udder conformation to IMIs. In particular, a poorly shaped udder which may be difficult for lambs to suck, may predispose the teats to bruises and lesions during active suckling events. Menzies and Ramanoon (2001) showed that poor udder shape lead to ineffective milk evacuation, and increased proneness to IMIs. The frequency of IMI predisposition was not clearly explained but irregular glands (i.e. deep and pendulous udder) with horizontally placed teats showed increased susceptibility to mastitis and high SCC was found in ewes that had pendulous udder (Casu et al., 2010; Gelasakis et al., 2012). This resulted to a reduction of machine-milkability in dairy ewes and caused high incidence of retained milk in the udder cistern due to consistent falling off of the milking clusters during milking. The importance of this in non-dairy ewes has not been well-established but suggests that pendulous udders could have secondary effects (e.g., insufficient milk and suboptimal growth rate) in suckling lambs. Furthermore, Huntley et al. (2012) discovered that poor udder conformation (with badly placed teats) in suckler ewes lead to high incidence of SCM with increased SCC, and subsequently affected lamb growth by 10% due to lower milk production.

Therefore, the selection of ewes with better udder conformation could limit susceptibility to IMIs and potentially improve lamb growth in flock production.

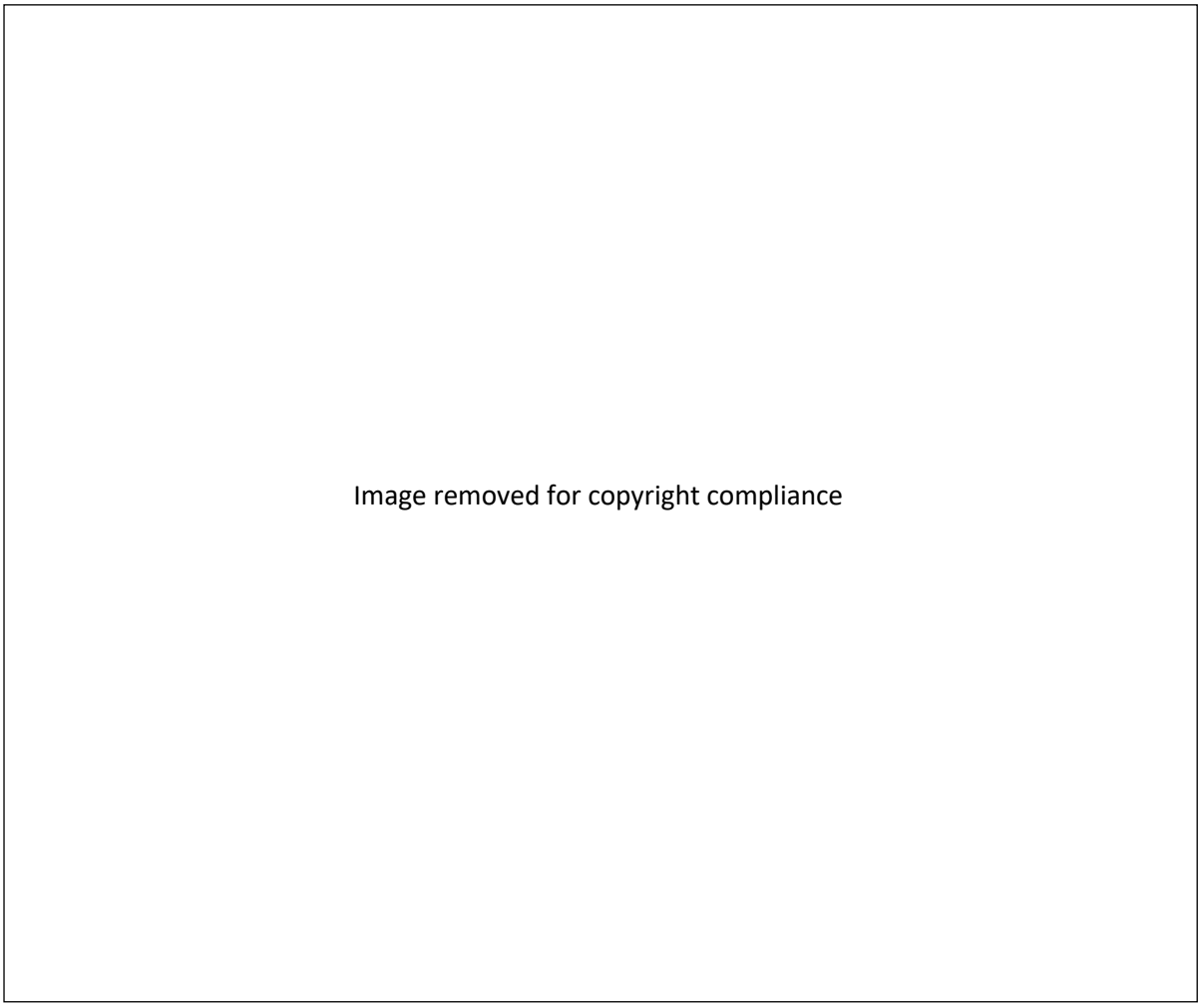

Figure 2.4: 1 (A) Optimal teat placement on udder and (B) Optimal udder depth in ewes (Green et al., 2016)

As highlighted in Figure 2.4 (A), the optimal teat angle score of 5 (45^{o} angle on the udder) has been associated with higher lamb weightgain (Casu et al., 2006; Green et al., 2016), which could be as a result greater milk production. Similarly, the teat angle score 5 was linked with lower risk of teat lesions compared with other teat scores which may be pointing further downward or forward (in suckling lambs) and potentially increasing the risk of acute mastitis (Green et al., 2016). The reason for this was suggested to be that, the teat exhibiting the less optimal angles are likely less protected by the non-woolly skin in the flank region and potentially exposed to climate and soil environments which may harbour infectious pathogens (e.g., Escherichia coli). However, teat score 1 is well suited for machine-milking (in dairy ewes)

but rare in the non-dairy ewes (Green et al., 2016). The udder depth position has been widely associated with milk production in ewes. Udder depth score 7, as seen in Figure 2.4 (B) has been described optimal for effective lamb suckling (Casu et al., 2006; Green et al., 2016) and possibly less prone to IMIs.

2.7.4 Importance of milk production on lamb growth

The relationship between lamb growth rate and milk production is strongest from birth to 4 weeks of lactation (Torres-Hernandez and Hohenboken, 1980; Treacher, 1983). Perinatal lamb mortality with estimated starvation and mismothering of about 34% in ewes has been associated with mastitis or lack of milk (Johnston et al., 1980). Numerous studies have shown such cases with links to IMIs causing reduced weight-gain in lambs (at 20 g/day), and up to 4 kg at weaning (Ahmad et al., 1992; Fthenakis and Jones, 1990; Larsgard and Vaabenoe, 1993). Many reports suggested that milk production is influenced by litter size (i.e. number of lambs), and ewes with more lambs produce more milk per lactation than single-bearing ewes but the lambs that survive through lactation are those sufficiently fed. However, CM has been found to cause pain in lactating ewes through the presence of mechanical hyperplasia (Dolan et al., 2000) which may deprive suckling lambs of adequate milk and culminating to poor lamb growth. Similarly, (McLennan et al., 2016) described the effects of mastitis in ewes which subsequently displayed changes in their facial expression during udder examination (by palpation) as responses to different levels of pain. This could affect lamb production through the reduction of the ewes' willingness to be suckled.

2.8 Summary

A number of factors influence udder health and the risk of IMI. IMI has been found to reduce milk production and may affect lamb growth rate through inadequate food for suckling lambs. Further, a sound udder that is free from any structural defects could provide no impediment to lambs' ability to suckle during lactation and reduce the risk of IMI. However, there is a paucity of information available for the incidence of SCM, its effect on milk production and lamb growth rates and the importance of udder conformation in non-dairy sheep.

Chapter 3

Material and Methods

3.1 Experimental design

The experiment was conducted at the LincolnSheep unit, Lincoln University, Canterbury, New Zealand with the approval from and in accordance with the Lincoln University Animal Ethics Committee, application No LUAEC 2017-17.

The lactation yield, milk nutrient composition, milk somatic cells counts, milk bacteriological analysis, lamb live weight gain and udder characteristics of 121 (predominantly Coopworth) twin-suckled ewes were assessed during lactation. Within 24 h of parturition, lamb weightwas recorded, and the lambs were tagged and matched to dam. Each week during lactation, ewes which had lambed in that week and their lambs were moved into a separate paddock, providing three cohorts of ewes, each with a similar lambing date. For each cohort at four weeks, eight weeks and twelve weeks post-parturition, ewes were assessed for lactation performance and udder characteristics (described below) in addition, ewe live weight and body condition score and lamb weights were recorded. Post-weaning, ewes were maintained on ryegrass pastures for the remainder of the summer as per normal farm practice until the subsequent mating (April) when the udder assessment was repeated to determine the incidence of udder deformations that did not self-resolve.

3.2 Ewe Udder Assessment

At each assessment time the following udder characteristics were recorded. The presence of lesions or inflammation on the teats and udder in addition to each teat and udder half palpated and scored for consistency on a scale of 1 to 5 and 1 to 7, for teats and udders, respectively, as described in Table 3.1. Teat length and width were measured using digital callipers (Jobmate digital callipers: model – J701 - 2702, Auckland, New Zealand).

Table 2.1: Scores related to physical characteristics of teats and udders following palpation. Scoring chart supplied by Kate Griffiths (Massey University)

Palpation score	Teat palpation	Udder palpation
1	Soft consistency	Diffuse soft consistency
2	Thickened teat orifice	Diffuse firm consistency
3	Hard consistency	Soft consistency with small lump(s) < 2 cm in size
4	Palpation of a dense, vertical cord in the center of the teat	Soft consistency with large lump(s) > 2 cm in size
5	Teat orifice obstruction	Firm consistency with small lump(s) < 2 cm in size
6	-	Firm consistency with large lump(s) > 2 cm in size
7	-	Diffuse hard consistency

Teat placement, udder depth, udder suspension and udder separation were assessed visually according to the scoring chart given in Figure 3.1. Teat placement was assessed on a scale of 1 to 5 with 1 being the downward pointing teats (and closer to the udder's lowest point) and 5 being the teats are farther away from the lowest point of the udder. Udder depth was recorded on a scale of 1 to 5 with 1 being the glands are hanging below the hock level and 5 being that the glands are hanging high and tight to the belly. Udder suspension was recorded on a scale of 1 to 5, with 1 being the udder width at attachment to the belly is transversely narrower than the depth while 5 being the attachment width is much larger than the udder depth. Degree of separation was recorded on a scale of 1 to 5 with 1 being that there is no visible division of the udder halves and 5 being that the glands are clearly divided into two halves. Udders were also scored for symmetry (Yes/No).

3.3 Milk production and somatic cell counts

Milk production was assessed using the oxytocin method as described by Bencini et al. (1992b) and the 4-hour milking method to estimate daily milk yield as described in previous studies (Afolayan et al., 2009; Hunter et al., 2015). At each time, each cohort of ewes were allocated into batches of 10 to ensure accurate timing of sampling between milkings. For each batch of 10 ewes, the lambs were removed and 5ml of milk that was the first to be removed from the teat was aseptically collected from each udder-half and stored at -80^{o}C for bacteriological assessment (described below).

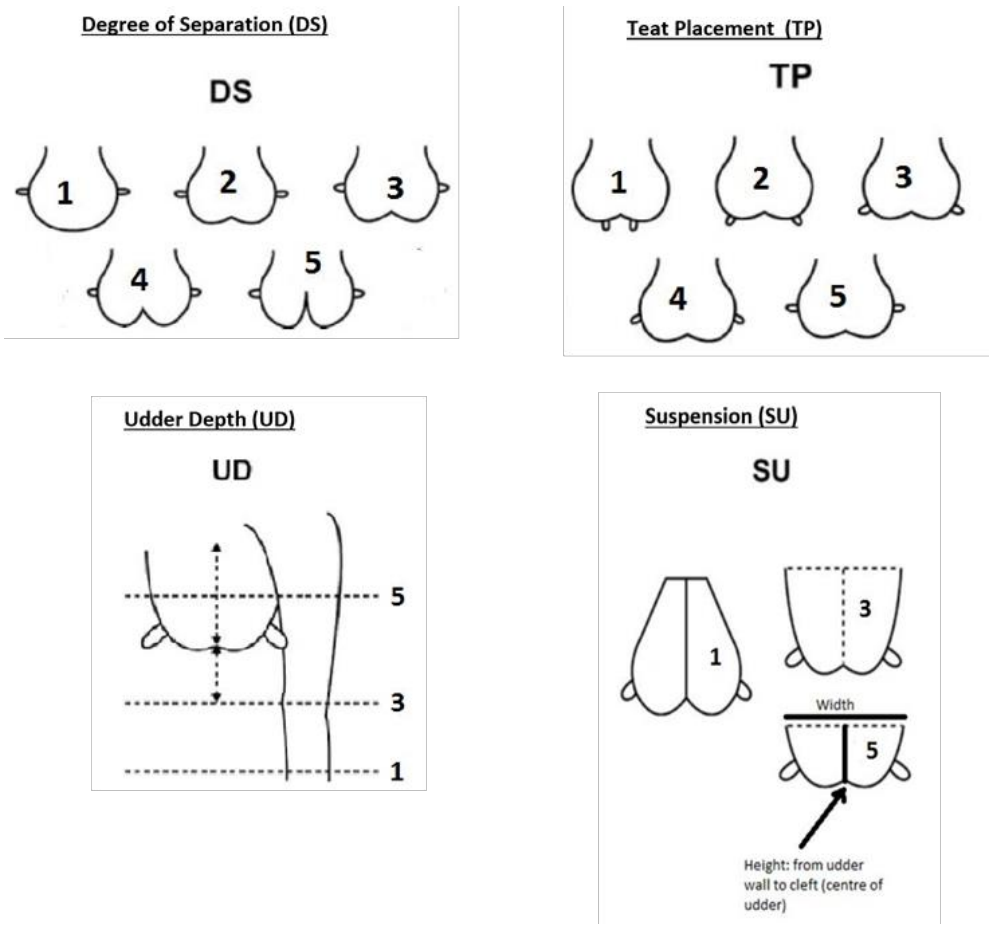

Figure 3.1: Examples of the visual scoring system for degree of separation (DS), teat placement (TP), Udder depth (UD) and udder suspension (SU) for ewes. Images and scoring chart supplied by Kate Griffiths (Massey University)

The lambs were first separated from their dams and kept in another paddock. Afterwards, the ewes were first milked using a milking machine (DeLaval Type DVP170/340/EF601516001-TJ Tumba, Sweden) to empty the udder and the time of this first milking was recorded (usually at 09:00am). After four hours (usually at 01:00pm) the ewes were administered with 1.0 mL of oxytocin injection (0.0167 mg/mL at 10 IU mL^{-1}, Kela N.Y Hoogstraten, Belgium/batch number - 26824A10) intramuscularly and after 1 minute, they were milked again using a herd testing sampler (C0180 and C0001, supplied by Livestock Improvement Corporation Ltd LIC; Christchurch, New Zealand) which had been calibrated for sheep. The weight of the sub-sample was multiplied using the formula: milk volume in grams ÷ nozzle size = milk litres/day to calculate milk production during the four-hour period. Sub-samples were preserved with bronopol (0.1%) and analysed at Livestock Improvement Corporation Ltd (LIC; Christchurch, New Zealand) to determine SCC, milk fat, protein and lactose by MilkoScan (Foss Electric,

Hillerød, Denmark). Following the second milking the lambs were returned to their dams and all the animals were returned to their allocated paddock.

3.4 Bacteriological Analysis

The aseptically collected samples (5 mL each) were kept at −80°C until the bacteriological analysis was conducted by Alex Grinberg, IVABS, Massey University, New Zealand. Briefly, 20 µl of each milk sample was plated on blood agar plates which were then incubated aerobically at 37°C and examined at 24 and 48 hours (Gelasakis et al., 2015). Cultures were then identified for bacterial colonies using Gram staining technique, catalase and coagulase tests.

3.5 Statistical Analyses

Data were analysed using GenStat 18 (VSN International Ltd: Hemel Hempstead, United Kingdom). Data were classified into above or below SCC threshold of 400,000 cells per mL (Arsenault et al., 2008; Huntley et al., 2012; Kern et al., 2013). The relationship between milk production, lamb liveweight gain, SCC and udder characteristics were analysed using analysis of variance (ANOVA), and the means were separated with Fisher's Least Significant Difference test at points of significance. Milk production data from one cohort of animals was not assessed at week 4 due to laboratory error and were subsequently removed from the analysis. At week 12, the sub-samples from 65 animals were insufficient for milk composition and SCC analysis, with subsequent comparisons an associations from these animals excluded from the results. One ewe displayed clinical symptoms of mastitis and was treated with antibiotics. Data from this ewe were excluded from the study due to inadequate milk production.

Chapter 4

Results

4.1 Milk production

Mean milk production and composition for all ewes at each time is given in Table 4.1. Overall, for all animals, milk production per 4 hours was greatest at the first milking week 4 of lactation, viz, 1.09 ± 0.04 litres which declined to 0.68 ± 0.02 litres by week 8 and 0.48 ± 0.01 litres by week 12. Milk fat yield per 4 hours was greatest at week 4 of lactation, viz, 63.80 ± 2.84 g which declined to 43.45 ± 1.48 g by week 8 and 36.66 ± 0.95 g by week 12. Milk protein yield per 4 hours was greatest at week 4 of lactation, viz, 54.37 ± 1.88 g which declined to 34.83 ± 1.03 g by week 8 and 26.03 ± 0.63 g by week 12. Milk lactose yield per 4 hours was greatest at week 4 of lactation, viz, 61.08 ± 1.94 g which declined to 36.75 ± 1.15 g by week 8 and 24.80 ± 0.61 g by week 12. Total milk solids yield per 4 hours was greatest at week 4 of lactation, viz, 184.9 ± 6.51 g which declined to 118.8 ± 3.63 g by week 8 and 90.6 ± 2.11 g by week 12.

4.2 Prevalence of high SCC and distribution

The mean SCC and distribution of animals above and below the threshold of 400,000 cells per mL and their associated milk volumes, milk fat yield, milk protein yield, milk lactose yield, milk solids yield per 4 hours and lamb liveweight gain for the previous four weeks at each time are given in Table 4.1. Overall, 20 ewes had elevated SCC at one or more time, and of these, none had elevated SCC at all three sampling times, five had elevated SCC on two occasions and 15 on one occasion, with corresponding incidences of SCM of 12.7%, 9.8% and 8.9% at weeks 4, 8 and 12, respectively.

At week 4 of lactation there was no difference between those ewes with SCC above the threshold of 400,000 cells per mL or those below the threshold for milk volume, milk fat yield, milk protein yield, milk lactose yield, total milk solids production or lamb liveweight gain ($P>0.05$ for all). At week 8 of lactation milk volume, milk fat yield, milk protein yield, milk lactose yield and total milk solids production was less in ewes with SCC above the threshold of 400,000 cells per mL than those with below the threshold ($P<0.05$ for all) but there was no difference in lamb liveweight gain ($P>0.05$). At week 12 of lactation there was no difference in milk volume, milk fat yield, milk protein yield, milk lactose yield and total milk solids

production or lamb liveweight gain in ewes with SCC above the threshold of 400,000 cells per mL compared with those with below the threshold ($P>0.05$ for all).

Table 3.1: Mean somatic cell count (SCC) and distribution of animals above and below the threshold of 400,000 cells/mL at weeks 4, 8 and 12 of lactation and previous sampling time for week 8 and 12 in relation to milk per 4 h and lamb liveweight gain for twin- suckled lamb per previous four-week period

Week	SCC threshold	Previous SCC threshold	n	SCC (x 10^6 cells ml^{-1})	Milk yield (liters per 4 h)	Milk fat (g per 4 h)	Milk Protein (g per 4 h)	Milk Lactose (g per 4 h)	Milk Total Solid (g per 4 h)	Lamb weight gain (kg per 4weeks)
4	Above		10	1.68	1.07 ± 0.10[a]	70.1 ± 7.56	57.5 ± 5.71	57.5 ± 5.80	191.3 ± 18.44	7.26 ± 0.56[a]
	Below		69	0.12	1.09 ± 0.04[a]	62.9 ± 3.07	53.9 ± 1.99	61.6 ± 2.07	183.9 ± 7.00	7.82 ± 0.20[a]
8	Above	All	10	3.06	0.52 ± 0.05[a]	35.4 ± 2.99[a]	28.4 ± 2.14[a]	27.4 ± 2.50[a]	94.4 ± 7.27[a]	7.22 ± 0.46[a]
		Above at week 4	4	2.92	0.50 ± 0.07	33.5 ± 5.55	29.0 ± 4.07	26.1 ± 3.17	91.8 ± 12.94	6.78 ± 0.81
		Below at week 4	6	3.16	0.53 ± 0.07	36.7 ± 3.69	28.1 ± 2.66	28.2 ± 3.79	96.1 ± 9.49	7.52 ± 0.57
	Below	All	92	0.11	0.70 ± 0.02[b]	44.3 ± 1.58[b]	35.5 ± 1.09[b]	37.8 ± 1.21[b]	121.4 ± 3.86[b]	7.30 ± 0.14[a]
		Above at week 4	5	0.09	0.78 ± 0.16	48.7 ± 8.99	39.6 ± 6.95	43.3 ± 8.89	135.8 ± 25.29	7.70 ± 0.68
		Below at week 4	60	0.11	0.69 ± 0.03	43.6 ± 1.80	34.9 ± 1.30	37.8 ± 1.40	120.1 ± 4.47	7.24 ± 0.15
12	Above	All	5	1.78	0.45 ± 0.03[a]	34.5 ± 3.07	24.7 ± 1.76	23.2 ± 1.97	85.4 ± 6.31	13.01 ± 2.03[a]
		Above at week 8	1	0.74	0.42	30.7	23.31	21.7	78.1	17.00
		Below at week 8	3	2.28	0.47 ± 0.06	34.5 ± 5.15	23.9 ± 2.72	24.8 ± 3.04	86.1 ± 10.92	10.32 ± 2.16
		Above at week 4+8	-	-	-	-	-	-	-	-
		Below at week 4+8	4	2.04	0.46 ± 0.04	35.5 ± 3.76	25.0 ± 2.23	23.6 ± 2.49	87.2 ± 7.79	12.01 ± 2.28
	Below	All	51	0.14	0.49 ± 0.01[a]	36.9 ± 1.01	26.2 ± 0.68	25.0 ± 0.64	91.1 ± 2.23	10.74 ± 0.57[a]
		Above at week 8	3	0.17	0.41 ± 0.02	32.9 ± 0.87	21.2 ± 1.65	21.3 ±1.36	77.7 ± 2.79	13.93 ± 0.32
		Below at week 8	30	0.13	0.49 ± 0.01	36.8 ± 1.34	26.1 ± 0.80	25.2 ± 0.64	91.2 ± 2.75	9.31 ± 0.70
		Above at week 4+8	-	-	-	-	-	-	-	-
		Below at week 4+8	30	0.13	0.49 ± 0.01	36.8 ± 1.34	26.1 ± 0.80	25.2 ± 0.64	91.2 ± 2.75	9.31 ± 0.70

For means of milk production and lamb liveweight gain within each sampling time for all samples above or below the threshold, those with different superscripts are significantly different (P<0.05)

4.3 Bacteriology

Of the 474 milk samples that were aseptically collected from the animals throughout the sampling periods, 158 samples were collected from both udder halves of the same animal in week 4 which consisted of 79 samples each from the left and right udder halves. Similarly, 204 milk samples (102 each from both udder halves) were collected in week 8, while 112 were collected in week 12 (56 each from both udder halves). Table 4.5 shows the overall result of the milk bacteriology throughout the sampling periods.

In week 4, 16% of the milk samples displayed the presence of coccus-shaped bacteria species, which consisted of 14 samples from the left udder (posteriorly), and 11 samples from the right udder. Pleomorphic-shaped bacteria species was also discovered in 4% of the samples that consisted of 3 samples each from both udder halves. A total of 4% of the samples displayed coco bacillus-shaped species which comprised of 1 milk sample from the left udder and 5 from the right. No cases of diplococcus-, streptococcus- and rod-shaped bacteria species were found in the left udder but 1, 3, and 4, respectively were displayed in the right udder. However, no bacterial growth (NG) was recorded in 63% of the samples which comprised of fifty-four samples on the left and forty-six from the right, while sample contamination was at 4% (three samples each from both udder halves).

In week 8, there was a decline in the presence of coccus-shaped bacteria species to 11%, which consisted of seven milk samples from the left gland and four samples from the right gland. Similarly, the presence of pleomorphic-shaped bacteria species also declined to 2% which consisted of one milk sample each from both udder halves. Coccobacillus- (1%) and diplococcus-shaped species (1%) occurred with one milk sample each from the left udder and none at the right. Streptococcus-shaped species was found at 1%, which was only found in the right udder (one milk sample) and none in the left. Rod-shaped bacteria species had a prevalence of 5%, which comprised of three milk samples from the left udder and two from the right. Further, NG was recorded in 86% of the samples which consisted of eighty-four samples on the left and ninety-two from the right, while contamination was at 2% (comprised of two samples from the left and none from the right).

In week 12, there was a further decline in the presence of coccus-shaped bacteria species to 3%, which consisted of two milk samples from the left udder and none from the right. Similarly,

there was no pleomorphic-, streptococcus-, rod-shaped bacteria species in any of the udder halves, but coco bacillus- (2%) and diplococcus-shaped species (2%) occurred with one milk sample each from the right udder and none in the left. Moreover, NG was recorded in 97% of the samples which comprised of fifty - five samples on the left and fifty - four from the right, while no sample contamination was present.

Taxonomic classification of the bacteria species was only possible for the samples which displayed the presence of *Staphylococcus aureus*. In total, three ewes displayed the presence of *S. aureus* which occurred in 1% of the milk samples in week 4 (comprising of two samples from the right udder), and 1% in week 8 (comprised of only one milk sample from the right udder) while none was discovered in week 12. The mean SCC, lamb weight gain, milk yield and composition of the *S. aureus* positive ewes are given in Table 4.2, with these characterized by high SCC at week 8 (1.97 x 10^6 cells mL^{-1}) and week 12 (1.07 x 10^6 cells mL^{-1}) compared with 0.27 x 10^6 cells mL^{-1} at week 4. There was also a declining LWG, with week 12 (5.70 kg per 4 weeks) being the lowest compared with week 8 (6.27 kg per 4 weeks) and week 4 (6.34 kg per 4 weeks). Similarly, milk yield for week 12 (0.41 litres per 4 h) was the lowest compared with week 8 and week 4 (0.59 litres per 4 h, 1.05 litres per 4 h, respectively).

Table 4.2: Mean somatic cell count (SCC), lamb liveweight gain, milk yield per 4 h, and milk composition for ewes that showed the presence of Staphylococcus aureus at weeks 4, 8 and 12 of lactation

Week	SCC (x 10^6 cells mL^{-1})	n	Lamb weight gain (kg per 4weeks)	Milk yield (litres per 4 h)	Milk fat (g per 4 h)	Milk Protein (g per 4 h)	Milk Lactose (g per 4 h)	Milk Total Solid (g per 4 h)
4	0.27	3	6.34	1.05	66.92	51.75	58.07	182.49
8	1.97	3	6.27	0.59	35.74	30.30	32.49	101.69
12	1.07	2	5.70	0.41	25.92	20.05	21.47	70.02

One ewe was excluded from week 12 due to insufficient milk for analyses

4.4 Association between udder characteristics and SCC

Mean log10 SCC for each of the udder characteristic scores are given in Table 4.3. Presence of lesions and/or inflammation was evident in 15.7% of the animals at one or more times. The greatest incidence was at week 4 and only 7.6% of these were present at subsequent sampling times. Overall, for the parameters assessed, significant associations with SCC were observed for teat placement (TP) and teat palpation at week 12 ($P = 0.002$ and $P=0.046$, respectively) although no association was recorded between TP and SCC at weeks 4 for 8 ($P >0.05$ for all). There were no significant associations between SCC and scores for udder palpation, udder symmetry, udder suspension, or degree of udder separation at any of the assessment times ($P>0.05$ for all).

Table 4.3: Mean log10 somatic cell counts (SCC) for each score for teat palpation, teat lesions, teat placement, udder palpation, udder lesions, udder symmetry, udder depth, udder suspension, and udder degree of separation at weeks 4, 8, and 12 of lactation

	Score or presence							
Week 4	1	2	3	4	5	No	Yes	P =
Teat Palpation	5.14 ± 0.070	5.16 ± 0.078	5.32	5.06 ± 0.131	-	-	-	0.97
n=	54	21	1	3	-	-	-	
Teat inflammation/lesion						5.13 ± 0.236	5.28 ± 0.236	0.42
n=						72	7	
Teat placement	5.45	5.30 ± 0.155	5.08 ± 0.060	5.09 ± 0.675	5.13 ± 0.209	-	-	0.44
n=	1	20	37	17	4	-	-	
Udder palpation	5.11 ± 0.064	5.20 ± 0.107	5.21 ± 0.104	5.21 ± 0.166	-	-	-	0.89
n=	50	24	2	3	-	-	-	
Udder inflammation/lesion						5.13 ± 0.055	5.24 ± 0.138	0.55
n=						72	7	
Udder symmetry						5.31 ± 0.127	5.09 ± 0.053	0.06
n=						20	59	
Udder depth	-	5.40 ± 0.131	5.18 ± 0.107	5.12 ± 0.064	5.12 ± 0.210	-	-	0.83
n=	-	2	21	50	6	-	-	
Udder suspension	5.22 ± 0.200	5.14 ± 0.076	5.11 ± 0.086	5.13 ± 0.118	5.78 ± 0.879	-	-	0.40
n=	3	31	26	17	2	-	-	
Udder degree of separation	5.13 ± 0.088	5.13 ± 0.072	5.15 ± 0.132	5.38 ± 0.385	-	-	-	0.84
n=	22	36	18	3	-	-	-	
Week 8								
Teat Palpation	5.09 ± 0.045	5.12 ± 0.120	4.76	-	-	-	-	0.73
n=	73	28	1	-	-	-	-	
Teat inflammation/lesion						5.10 ± 0.047	4.97 ± 0.107	0.62
n=						99	3	
Teat placement	5.11 ± 0.103	5.06 ± 0.068	5.17 ± 0.097	5.09 ± 0.131	-	-	-	0.82
n=	22	49	23	8	-	-	-	
Udder palpation	5.08 ± 0.048	5.02 ± 0.116	5.24 ± 0.112	5.65 ± 0.464	-	-	-	0.08
n=	80	15	3	4	-	-	-	
Udder inflammation/lesion						5.07 ± 0.044	5.44 ± 0.321	0.06
n=						96	6	
Udder symmetry						5.19 ± 0.141	5.08 ± 0.049	0.44
n=						12	90	
Udder depth	-	5.10 ± 0.400	5.12 ± 0.086	5.09 ± 0.052	5.12 ± 0.174	-	-	0.99
n=	-	2	10	77	13	-	-	
Udder suspension	5.22 ± 0.619	5.05 ± 0.044	5.04 ± 0.062	5.10 ± 0.080	5.26 ± 0.244	-	-	0.63
n=	5	17	37	30	13	-	-	
Udder degree of separation	5.28 ± 0.148	5.05 ± 0.076	5.16 ± 0.078	4.96 ± 0.787	4.98 ± 0.085	-	-	0.23
n=	20	38	20	14	10	-	-	
Week 12								
Teat Palpation	5.16 ± 0.057[a]	5.13 ±	6.13[b]	-	-	-	-	0.04
n=	48	7	1	-	-	-	-	
Teat inflammation/lesion						5.17 ± 0.056	5.16 ± 0.078	0.94
n=						53	3	
Teat placement	-	6.55[b]	5.09 ± 0.043[a]	5.16 ± 0.084[a]	5.26 ± 0.170[a]	-	-	0.00
n=		1	23	24	8			
Udder palpation	5.17 ± 0.053	-	-	-	-	-	-	-
n=	56	-	-	-	-	-	-	
Udder inflammation/lesion						5.17 ± 0.053	-	-
n=						56	-	
Udder Symmetry						5.09 ± 0.047	5.19 ± 0.061	0.50
n=						8	48	
Udder depth	-	-	5.07 ± 0.178	5.21 ± 0.062	5.11 ± 0.117	-	-	0.66
n=	-	-	3	38	15	-	-	
Udder Suspension	5.01 ± 0.162	5.18 ± 0.109	5.24 ± 0.087	5.21 ± 0.123	4.93 ± 0.107	-	-	0.43
n=	3	10	23	13	7	-	-	
Udder degree of separation	5.12 ± 0.071	5.15 ± 0.086	5.23 ± 0.122	5.12 ± 0.135	5.27 ± 0.192	-	-	0.93
n=	9	18	18	8	3	-	-	

Within rows means with different superscripts are significantly different (P<0.05)

4.5 Associations between udder scores and milk production

Mean milk yield for each of the udder characteristic scores is given in Table 4.4. For the traits of teat length (TL), teat width (TW), udder palpation, teat palpation, udder symmetry and udder suspension there was no association with milk yield at any time ($P>0.05$ for all). Milk yield was associated with UD ($P = 0.01$) at week 4, with UD score 3 the greatest viz, 1.27 ± 0.070 litres compared with UD score 4, viz, 1.04 ± 0.041 litres but no such associations were observed at weeks 8 or 12. For udder degree of separation (DS) at week 8, DS score 4 had the greater milk yield than DS score 3 ($P = 0.008$) although this was not observed at weeks 4 and 12 ($P>0.05$). There was no association between the presence of teat or udder lesions and milk yield at any sampling time ($P>0.05$).

Table 4.4: Mean milk production (litres per 4 h) for each score for teat palpation, teat lesion, teat placement, udder palpation, udder lesion, udder symmetry, udder suspension and udder degree of separation at wees 4, 8, and 12 of lactation

	Score or prese nce							
Week 4	**1**	**2**	**3**	**4**	**5**	**No**	**Yes**	**P =**
Teat Palpation	1.07 ± 0.043	1.14 ± 0.075	1.21	1.13 ± 0.073	-	-	-	0.794
n=	54	21	1	3	-	-	-	
Teat inflammation/lesion						1.08 ± 0.037	1.18 ± 0.134	0.424
n=						72	7	
Teat placement	1.47	1.11 ± 0.069	1.10 ± 0.050	1.04 ± 0.067	0.96 ± 0.304	-	-	0.613
n=	1	20	37	17	4	-	-	
Udder palpation	1.06 ± 0.043	1.18 ± 0.064	0.87 ± 0.003	1.03 ± 0.258	-	-	-	0.316
n=	50	24	2	3	-	-	-	
Udder inflammation/lesion						1.10 ± 0.037	1.00 ± 0.140	0.411
n=						72	7	
Udder symmetry	-	-	-	-	-	1.00 ± 0.069	1.12 ± 0.041	0.127
n=	-	-	-	-	-	20	59	
Udder depth	-	0.79 ± 0.175[a]	1.27 ± 0.070[b]	1.04 ± 0.041[a]	0.97 ± 0.130[a]	-	-	**0.010**
n=	-	2	21	50	6	-	-	
Udder Suspension	1.12 ± 0.347	1.08 ± 0.049	1.13 ± 0.072	1.06 ± 0.061	0.94 ± 0.422	-	-	0.919
n=	3	31	26	17	2	-	-	
Udder degree of separation	1.13 ± 0.058	1.06 ± 0.052	1.14 ± 0.094	0.91 ± 0.030	-	-	-	0.594
n=	22	36	18	3	-	-	-	
Week 8								
Teat Palpation	0.69 ± 0.026	0.65 ± 0.034	0.95	-	-	-	-	0.335
n=	73	28	1	-	-	-	-	
Teat inflammation/lesion						0.68± 0.022	0.50 ± 0.047	0.145
n=						99	3	
Teat placement	-	0.67 ± 0.039	0.71 ± 0.034	0.68 ± 0.041	0.54 ± 0.044	-	-	0.237
n=	-	22	49	23	8	-	-	
Udder palpation	0.69 ± 0.024	0.71 ± 0.050	0.46 ± 0.032	0.48 ± 0.062	-	-	-	0.060
n=	80	15	3	4	-	-	-	
Udder inflammation/lesion						0.68 ± 0.021	0.73 ± 0.133	0.568
n=						96	6	
Udder symmetry	-	-	-	-	-	0.55 ± 0.048 [a]	0.70 ± 0.023 [b]	**0.028**
n=	-	-	-	-	-	12	90	
Udder depth	-	0.58 ± 0.021	0.66 ± 0.068	0.70 ± 0.025	0.60 ± 0.047	-	-	0.418
n=	-	2	10	77	13	-	-	
Udder Suspension	0.51 ± 0.045	0.75 ± 0.063	0.69 ± 0.035	0.65 ± 0.033	0.68 ± 0.066a	-	-	0.208
n=	5	17	37	30	13	-	-	
Udder degree of separation	0.60 ± 0.039[a]	0.70 ± 0.034[a]	0.61 ± 0.040[a]	0.71 ± 0.056[ab]	0.86 ± 0.082[b]	-	-	**0.008**
n=	20	38	20	14	10	-	-	
Week12								
Teat Palpation	0.49 ± 0.012	0.47 ± 0.033	0.42	-	-	-	-	0.639
n=	48	7	1	-	-	-	-	
Teat inflammation/lesion						0.49 ± 0.011	0.40 ± 0.015	0.076
n=						53	3	
Teat placement	-	0.49	0.49 ± 0.018	0.47 ± 0.017	0.48 ± 0.030	-	-	0.892
n=	-	1	23	24	8	-	-	
Udder palpation	0.48	-	-	-	-	-		-
n=	56	-	-	-	-	-		
Udder inflammation/lesion						0.48	-	-
n=						56	-	
Udder symmetry	-	-	-	-	-	0.49 ± 0.031	0.48 ± 0.012	0.842
n=	-	-	-	-	-	8	48	
Udder depth	-	-	0.57 ± 0.075	0.48 ± 0.012	0.47 ± 0.025	-	-	0.141
n=	-	-	3	38	15	-	-	
Udder Suspension	0.48 ± 0.043	0.53 ± 0.029	0.47 ± 0.014	0.47± 0.018	0.49 ± 0.049	-	-	0.387
n=	3	10	23	13	7	-	-	
Udder degree of separation	0.46 ± 0.017	0.47 ± 0.021	0.49 ± 0.022	0.50 ± 0.026	0.58 ± 0.041	-	-	0.231
n=	9	18	18	8	3	-	-	

Within rows means with different superscripts are significantly different (P<0.05)

4.6 Association between SCC, milk production and lamb growth

Mean weights of each of the twin lambs were 4.57 ± 0.087 kg, 12.3 ± 0.227 kg, 20.2 ± 0.302 kg and 22.1 ± 0.445 kg at birth, week 4, week 8 and week 12, respectively. The associations between either the milk yield or SCC and lamb weight gain in the four weeks prior to sampling are given in Figure 4.1 (a - f). Overall, regardless of sampling time, there was a very weak associations between both milk yield and SCC and lamb growth but none of the regression was statistically significant. At week 4, the relationship between milk yield and lamb growth was y = 0.0555x + 0.6605 (r^2 = 0.0 861) and between SCC and lamb growth was y = -6207.5x + 369027 (r^2 = 0.0002) (P>0.05 for both). At week 8, the relationship between milk yield and lamb growth was y = 0.0092x + 0.6115 (r^2=0.0033) and between SCC and lamb growth was y = - 82467x + 999683 (r^2 = 0.0068) (P>0.05 for both). At week 12, the relationship between milk yield and lamb growth was y = -0.0038x + 0.5249 (r^2=0.0354) and between SCC and lamb growth was y= 3384.1x + 245904 (r^2 = 0.0006) (P>0.05 for both).

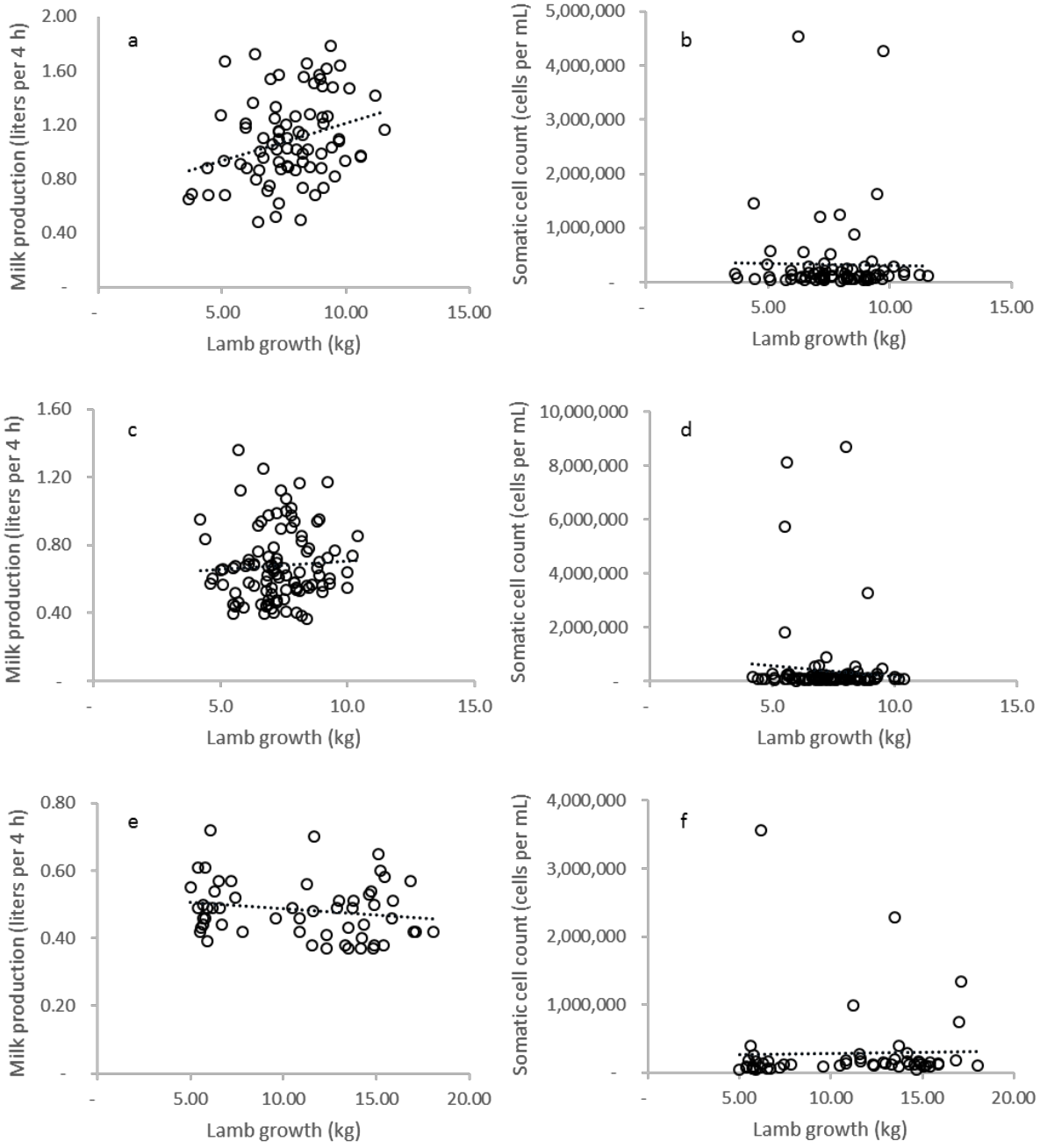

Figure 4.1: Relationship between lamb growth during the previous 4 weeks and milk production (litres per 4 h) (a, c, e) and somatic cell count (b, d, f) of ewes at week 4 (a, b), week 8 (c, d) and week 12 (e, f) of lactation

Table 4.5: Milk bacteriology for the 474 milk samples collected from both udder halves (right and left) for weeks 4, 8 and 12

Udder side	Week	n	Contamination	No growth	Number of colonies				S. aureus	Cocci				Yeast cell	pleomorphic		Coco bacilli		Rods	
					1	2	3	>3		+	-	In chains	Diplococci Diplococci		+	-	+	-	+	-
Left																				
	4	79	3	54	1	3	1	5	-	14	4	-	-	-	3	-	1	-	-	-
	8	10	2	84	1	1	2	1	-	7	1	-	1	-	1	1	1	-	3	1
	12	56		55	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Right																				
	4	79	3	46	1	4	1	13	2	11	2	3	1	-	3	1	5	-	4	-
	8	10		92	5	3	1	1	1	4	-	1	-	-	1	-	-	2	2	-
	12	56		54	2	-	-	-	-	2	-	-	1	1	-	-	1	-	-	-

4.7 Association between udder characteristics and lamb growth

Associations between udder characteristics and lamb liveweight gain are given in Table 10. There were no associations with lamb liveweight gain for the udder characteristics of SU, DS and udder symmetry at any of the sampling periods (P>0.05 for all) except at week 4 (UD: P = 0.02) where mean lamb liveweight gain of 7.32 kg for UD score 4 was less than UD scores 2, 3 and 5 (8.95 kg, 8.35 kg and 8.79 kg, respectively), and at week 8 for TP (P = 0.04) where those ewes with a TP of 3 had lamb weight gain of 7.28 kg compared with 8.95 kg, 8.35 kg and 8.79 kg for TP scores of 1, 2 and 5, respectively.

Table 4.6: Mean lamb liveweight gain (kg) per lamb in the previous four weeks for each score for teat palpation, teat lesion, teat placement, udder palpation, udder lesions, udder depth, udder suspension, and udder degree of separation at weeks 4, 8 and 12 of lactation

	Score or presence							
Week 4	1	2	3	4	5	No	Yes	P =
Teat Palpation	7.66 ± 0.233	8.16 ± 0.351	5.95	7.09 ± 0.215	-	-	-	0.387
n=	54	21	1	3	-	-	-	
Teat inflammation/lesion						7.71 ± 0.196	8.10 ± 0.659	0.557
n=						72	7	
Teat placement	10.15	7.75 ± 0.432	7.68 ± 0.263	7.76 ± 0.395	7.65 ± 0.629	-	-	0.716
n=	1	20	37	17	4	-	-	
Udder palpation	7.79 ± 0.223	7.59 ± 0.385	6.68 ± 0.675	8.87 ± 0.956	-	-	-	0.495
n=	50	24	2	3	-	-	-	
Udder inflammation/lesion						7.64 ± 0.194	8.87 ± 0.601	0.062
n=						72	7	
Udder symmetry						7.50 ± 0.410	7.83 ± 0.210	0.455
n=						20	59	
Udder depth	-	8.95 ± 1.65^{ab}	8.35 ± 0.380^{b}	7.32 ± 0.212^{a}	8.79 ± 0.682^{b}	-	-	**0.021**
n=	-	2	21	50	6	-	-	
Udder suspension	9.10 ± 0.964	7.87 ± 0.256	7.61 ± 0.409	7.61 ± 0.342	6.70 ± 0.450	-	-	0.527
n=	3	31	26	17	2	-	-	
Udder degree of separation	7.79 ± 0.348	7.58 ± 0.289	7.81 ± 0.385	9.03 ± 0.802	-	-	-	0.541
n=	22	36	18	3	-	-	-	
Week 8								
Teat Palpation	7.28 ± 0.156	7.26 ± 0.249	8.9	-	-	-	-	0.476
n=	73	28	1	-	-	-	-	
Teat inflammation/lesion						7.26 ± 0.134	8.23 ± 0.410	0.213
n=						99	3	
Teat placement		7.17 ± 0.237^{a}	7.28 ± 0.176^{a}	7.02 ± 0.328^{a}	8.53 ± 0.399^{b}	-	-	**0.040**
n=		22	49	23	8	-	-	
Udder palpation	7.38 ± 0.151	7.18 ± 0.296	6.60 ± 0.577	6.48 ± 0.734	-	-	-	0.427
n=	80	15	3	4	-	-	-	
Udder inflammation/lesion						7.27 ± 0.136	7.70 ± 0.513	0.439
n=						96	6	
Udder symmetry						7.20 ± 0.393	7.20 ± 0.140	0.799
n=						12	90	
Udder depth	-	9.15 ± 0.150	7.47 ± 0.470	7.29 ± 0.154	6.88 ± 0.234	-	-	0.145
n=	-	2	10	77	13	-	-	
Udder suspension	8.56 ± 0.470	7.39 ± 0.366	7.48 ± 0.239	6.87 ± 0.194	7.12 ± 0.274	-	-	0.064
n=	5	17	37	30	13	-	-	
Udder degree of separation	7.50 ± 0.254	6.83 ± 0.200	7.69 ± 0.298	7.41 ± 0.417	7.68 ± 0.427	-	-	0.088
n=	20	38	20	14	10	-	-	
Week 12								
Teat Palpation	10.82 ± 0.590	10.86 ± 1.667	17.1	-	-	-	-	0.329
n=	48	7	1	-	-	-	-	
Teat inflammation/lesion						10.84± 0.579	12.66 ± 1.152	0.464
n=						53	3	
Teat placement	-	6.2	10.88 ± 0.862	10.76 ± 0.850	12.25± 1.518	-	-	0.55
n=		1	23	24	8			
Udder palpation	10.94 ± 0.553	-	-	-	-	-	-	-
n=	56	-	-	-	-	-	-	
Udder inflammation/lesion						10.94 ± 0.553	-	-
n=						56	-	
Udder Symmetry						13.53 ± 1.369	10.51 ± 0.585	0.055
n=						8	48	
Udder depth	-	-	11.73 ± 2.930	11.51 ± 0.662	9.34 ± 1.024	-	-	0.217
n=	-	-	3	38	15	-	-	
Udder Suspension	12.62 ± 3.049	11.54 ± 1.078	11.62 ± 0.959	10.20 ± 1.037	8.51 ± 1.343	-	-	0.396
n=	3	10	23	13	7	-	-	
Udder degree of separation	10.79 ± 1.343	10.65 ± 0.991	10.68 ± 1.077	10.75 ± 1.279	15.23 ± 0.897	-	-	0.502
n=	9	18	18	8	3	-	-	

Within rows means with different superscripts are significantly different (P<0.05)

Chapter 5

Discussion & conclusion

Critical examination was conducted on the aspects of SCC, milk production and lamb growth in relation to udder characteristics and prevalence of mastitis in crossbred ewes using a combination of linear udder scoring and measurements. Similar methods have been evaluated in dairy ewes (Casu et al., 2006; Casu et al., 2010; De la Fuente et al., 1996). Casu et al. (2006) demonstrated a high level of repeatability with the same method across lactations and also speculated that similar effects may be present in suckler ewes. Further, Huntley et al. (2012) reported that poor udder characteristics and high SCC were linked to IMIs and subsequent lamb growth. Moreover, milk bacteriology, which is a gold standard for evaluating milk pathogens, mastitis and other IMIs, has been repeated in the current study to corroborate the aforementioned measurements, and to show the relationship among SCC, milk production, udder characteristics and mastitis in crossbred ewes. Although there have been previous studies on mastitis, there are few reports on SCC and its association with lamb production in crossbred ewes.

There are some limitations to interpretation of results in the current study. The sample size in this study was relatively small, being 121 ewes and 242 lambs. Further, some milk production records were omitted, on one occasion, due to conditions outside the experimental procedure as a result of laboratory error and on another occasion, due to the unforeseen complication of insufficient milk production for appropriate sub-sample to be collected from the herd-testing equipment and subsequent analyses. The profile of milk production, with the greatest milk yield at week 4 was as expected, reducing to less than 0.5 kg/d by week 12, but it was not anticipated that such a decline would result in an insufficient sample being collected. As such, given the limitations within this dataset, this can be considered a preliminary investigation into the relationship between SCC, milk production, and lamb growth in crossbred ewes, and the incidence of SCM, which has not previously been widely reported in NZ flocks (Peterson et al., 2017).

Generally, the incidence of high SCC across the sampling periods were inconsistent. Over 16% animals showed high SCC above the threshold of 400,000 cells per mL, and only a few had elevated SCC on more than one occasion. Antibiotic treatment was administered to only one ewe that expressed a clinical sign of “hard udder” (Peterson et al., 2017) at week 4, the animal was separated from the flock and subsequently removed from the study due to insufficient milk production. Previous studies have

associated hard udder i.e., indurative mastitis with maedi visna virus, which symptomatically result in breathing difficulty or nasal discharges, and can horizontally be transmitted across flock or vertically to suckling lambs via the ingestion of infected colostrum (Green et al., 2016; Heaton et al., 2013)**.** However, in the current study, the ewe did not display any plausible symptom and the lambs which could have given some indications died few days postpartum.

Milk bacteriology in the current study revealed a 5% presence of cocci-shaped bacteria species (being the highest), as affirmed by many reports that linked Staphylococcus species as the dominant agents of IMIs (Gelasakis et al., 2015). More importantly, the presence of Staphylococcus species in the current study was also predicated by the high mean SCC (Table 4.2) at weeks 8 and 12 which is in agreement with a Scottish study (Hariharan et al., 2004) that linked > 1 x 10^6 cells mL^{-1} SCC to *S. aureus*. However, surprisingly, all the ewes with high SCC in the current study were *S. aureus*- negative. This may be due to the insufficient milk production for SCC analysis or that most of the animals portrayed sound udder health which showed a reducing prevalence of udder and teat lesion/inflammation which was 18%, 9%, and 5% at weeks 4, 8, and 12 respectively, culminating in over 90% healthy udders when re- evaluated at mating. The isolation of *S. aureus* was inconsistent across the sampling periods as no individual (from the *S. aureus* positive ewes) displayed the bacteria more than once. Further, a majority of milk samples throughout the sampling periods showed no bacterial growth, with the greatest number at week 12 being 97% compared with weeks 4 (63%) and 8 (86%). This contrasts with a previous study (Smith et al. 2011) that recorded only 5% of samples with no bacterial growth. Similarly, there was also a contrasting 23% streptococcus- shaped bacteria species in the same study as opposed to the current study which only 1% was discovered. Also, at around 1% prevalence of *S. aureus* from the assessed milk samples in the current study, the incidence was considerably lower than that recorded in a Swedish study (Persson et al., 2017) that reported a 9% prevalence, with a corresponding high SCC > 400,000 per cells mL. Overall, these results indicate some levels of infection but was relatively low.

Previous studies have indicated that mastitis is a disease of high economic importance and one of the most important disease affecting sheep welfare across different ranges of sheep production and management systems in Europe (Berg et al., 2014; Gelasakis et al., 2015). This report encompasses the dairy and non-dairy ewes but recent reports suggested that the udder of suckler ewes is only assessed once a year (after weaning) or rarely done (for about 5

seconds) on many farms in NZ and Australia, as opposed to the frequent detection and management of mastitis in dairy ewes. Similarly, the reduction of milk yield (and composition) of the ewes that were positive for *S. aureus* (Table 4.2) agreed with previous studies (Arsenault et al., 2008; Moroni et al., 2007) although the impact on lamb growth rate appeared marginal, possibly reflecting the availability of pasture, hence, a lesser dependency on milk for growth.

Overall, the incidence of elevated SCC observed here compares favourably with the previously reported incidences of CM in crossbred sheep in NZ of around 5% with subclinical infections estimated to be three-fold (Peterson et al., 2017; Rattray et al., 1982). However, many studies have described the incidence of elevated SCC as dynamic and may differ depending on the time of measurement. In the current study all animals were stratified by lambing date and assessed at a similar time in lactation, and as such, the environmental conditions that may have potentiated exposure to IMI-causing pathogens (Klaas and Zadoks, 2017) may not have been present equally across each cohort, making comparison of the incidence in relation to stage of lactation more difficult to assess. Interestingly, high SCC was not generally associated with low milk production. At both weeks 4 and 12 those which were above the 400,000 cells per mL threshold showed no effect in milk production. In contrast, at week eight, those with SCC above the threshold appeared to have their milk production reduced by 25%. However, this was not reflected in differences in lamb growth. Although this needs to be repeated across different environments, the results of the current study indicate that the greatest impact of SCM on milk production may be around mid-lactation. However, a majority of ewes did not, at any time, display elevated SCC or show any signs of udder or teat lesion or inflammation, suggesting that the incidence of elevated SCC in crossbred ewes may be relatively low. Further to this, the animals were provided with ad-libitum access to pasture, which is supported in some reports which established the ability of well-nourished ewes to fight infections (Grant et al., 2016).

Overall, the udder characteristic scores which were reported here were poor indicators for both udder health and milk quality. Although several significant associations were discovered, these were inconsistent across sampling periods and did not provide a convincing predictor of potential SCC or milk production. In one instance, this was surprising as UD has been reported to have a strong relationship with milk production in ewes (Casu et al., 2000; Labussière, 1988). This was only apparent in the current study, as ewes with UD score 3 had heavier lambs at

weaning compared with UD score 4 which is in contrasts with (Casu et al., 2006; Green et al., 2016) that reported UD score 4 as optimal. There was lack of association between udder palpation scores and SCC, reflecting that very few animals had lumps. In another instance, the ewes in this study mostly had teats at TP score 3 and 4 which recorded lower SCC and rare lesions which is in agreement with previous reports of lower propensity of teat lesions at TP score 3 due to the optimal teat angle of 45° to the udder (Cooper, 2011; Green et al., 2016).

This study revealed a very week association between lamb liveweight gain and milk production which only occurred at week 4 but none of the regression was statistically significant. This agreed with the study of (Robinson et al., 1968) where lamb growth rates were associated with milk production at early but not late lactation. The udder and teat assessment of the ewes at subsequent mating period revealed that over 90% of udder defects had resolved, corroborating the reasons for 97% "no bacterial growth" for the milk bacteriology at week 12. Although there is no report on any direct health or growth implication in lambs consuming milk from IMI-infected ewes apart from reduction in milk yield (caused by IMI), resulting in starvation, mis-mothering and exposure to inclement weather which has been widely reported to affect lamb growth or cause mortality (Green et al., 2016; Jordan and Le Feuvre, 1989). Similarly, no report have suggested that high SCC milk may hinder or affect lambs growth and development. However, some studies have established other IMI causing bacteria like the *Mannheimia species*, which have been found to cause mastitis in sheep with a similar prevalence as the *S. aureus* (Omaleki et al., 2011) which was found in the current study. *Mannheimia haemolytica* were discovered at the nasopharyngeal region in healthy lambs, thereby causing mastitis and affecting milk production (Watkins and Jones, 1992). In contrast, the current study did not find *M. haemolytica* in the milk samples and the lambs were not evaluated for bacterial species.

The 121 ewes displayed over 90% healthy udders at mating, this could be linked with the sheep management system, being a common practice in NZ. The animals were kept in uncrowded open paddocks, where adequate pasture was made available while the study was conducted in the summer months after lambing. This may suggest that the environment provided a state of good hygiene (as shown in the current study), with over 80% of the ewes displayed low incidence of SCC across lactation. This is in tandem with the reports of (Gelasakis et al., 2015; Grant et al., 2016; Green et al., 2016) which described good hygiene, adequate nutrition, and

well-spaced housing as preventive methods of reducing IMIs. Similarly, as described in chapter 3, ewes and lambs were kept together throughout the study and this was also mentioned by these authors to make the animals acclimatize to their surrounding bacteria species easily, and this may give some insights about the bacteria species in the paddock area. However, the actual bacteria species in the paddocks were not evaluated throughout the study.

Breed resistance to mastitis has been identified in some English studies to limit subsequent incidences of mastitis with genomic technology (SRUC, 2015). This was hypothesised in Texel sheep breed, and preliminary reports suggested a link between mastitis and udder scores. A subsequent report found some relationships between mastitis and lamb weights (with a negative phenotypic correlation of −0.15 and −0.30), and it was inferred that higher level of the infection will result in lighter lambs. Although the breeds in the current study are predominantly Coopworth, this being the first mastitis evaluation in the flock, and showing a low incidences of the infection, it remains possible that the genotype of the animals used here may have contributed to the low incidence of elevated SCC. However, with this in mind, the incidences reported here were in line with previous reports on NZ pastures with a different breed (Peterson et al., 2017), implying a greater role of the environment rather than genotype in the incidence of IMI infections.

For future consideration, the lack of consistent association between udder characteristic scores, milk production and SCC in this study affords little basis from which recommendations on optimal udder characteristics can be made, thus, rendering the factors used in the current unimportant as pointers of mastitis in non-dairy ewes. However, it is evident that the suitability of these assessment methods needs to be further evaluated in a larger flock, and a more complete dataset to sufficiently validate the findings from the current study. Consideration needs to be given to methods of milk collection and SCC analysis, to avoid omission of samples. Although some other bacteria species were present in the current study giving indications of other plausible infections, a better understanding of these species is required. There may be reasons to include analyses of lamb-to-ewe transmission and the environmental risk factors, for example the bacteria species in the surrounding pasture, which may give rise to mastitis.

In conclusion, this study displayed the incidence of elevated SCC in about 16% of the ewes, varied across lactation, and showed an apparent ability of ewes to self-resolve infections, with reducing SCC towards the end of lactation in week 12. This was evident with the declining appearance of udder defects (i.e., inflammations/lesions) which peaked at week 4 (17.7%) and was the least at week 12 (5.4%), while over 90% of these disappeared post-weaning. It would be of interest to determine how many of these cases may be repeated in subsequent lactations. However, the high SCC gave indications of SCM but the impact on milk production was only obvious in week 8 and lamb growth was not affected. Furthermore, palpation of the udder and visual scoring for depth, degree of separation, suspension and symmetry did not appear to be a useful predictor of either SCC or milk production. Although the presence of *S. aureus* was established, this only occurred in a small number of cases, showing that it is not the sole cause of IMI. However, breed/genetic vigour, time of the day, or environmental factors were not considered for the current study and as such, might have implications on the interpretation of some of the presented data.

Chapter 6

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