Genome-wide association study for skin thickness and skin temperature traits in FocusPrime^{TM} New Zealand sheep.

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Abstract

This study investigated the genetic basis of skin traits in New Zealand FocusPrime sheep and their implications for lamb survival, with a particular focus on skin thickness and temperature, both of which play critical roles in the thermoregulation and overall survivability of lambs during the early stages of life. The primary objective was to identify genetic markers associated with these skin traits and to understand their heritability and correlation, thereby providing insights that could inform selective breeding programs aimed at improving lamb survival rates. The research employed a comprehensive genome-wide association analysis to analyse a large dataset of New Zealand FocusPrime sheep, meticulously measuring skin thickness and skin temperature, and correlating these phenotypic traits with genotypic data. The study found significant heritability estimates for both skin thickness and skin temperature, suggesting that these traits are genetically controlled and can be effectively targeted in breeding strategies. Specifically, the heritability of skin thickness was found to be high (values that exceed 0.40), indicating that a considerable proportion of the variation in this trait is due to genetic differences among individuals. Similarly, the heritability of skin temperature was also significant, underscoring the potential for genetic improvement through selective breeding. A key finding of this study was the negative correlation between skin thickness and skin temperature. Thicker skin, while beneficial in providing a barrier against environmental stressors, tends to be associated with lower skin temperatures, indicating a complex interplay in the thermoregulatory mechanisms of lambs. This finding highlights the need for a balanced approach in selective breeding, where both traits must be considered to optimize lamb survival. Breeding programs that focus solely on increasing skin thickness may inadvertently affect the thermoregulatory efficiency of lambs, thereby underscoring the importance of a holistic breeding strategy. The genome wide association study identified several significant genetic markers associated with skin thickness and skin temperature, providing valuable targets for future genetic selection. These markers offer a promising avenue for improving lamb survival through genetic means. The study also delves into the practical applications of these findings, suggesting that by incorporating these genetic markers into a breeding program, it is possible to enhance the resilience of lambs against harsh environmental conditions, thereby improving animal welfare and economic outcomes for sheep farmers. This research contributes to a broader understanding of the genetic factors influencing lamb survival, a topic of critical importance given the economic and welfare implications of lamb mortality. The findings have the potential to transform breeding practices in the New Zealand sheep industry, moving towards more scientifically informed strategies that enhance the overall sustainability and profitability of sheep farming. The implications of this research extend beyond New Zealand, offering insights that can be applied to sheep populations globally. Future research should continue to refine the genetic associations identified in this study and explore their practical implementation in diverse sheep populations. Further studies could also investigate the interaction of these genetic markers with environmental factors, providing a more comprehensive understanding of the determinants of lamb survival. By integrating genetic analysis with practical breeding strategies, there is significant potential to reduce lamb mortality rates and improve the resilience of sheep to environmental challenges. In conclusion, this study provides a robust framework for understanding the genetic basis of key skin traits in New Zealand sheep and their impact on lamb survival. The identification of heritable genetic markers associated with skin thickness and skin temperature paves the way for targeted breeding programs that can enhance lamb resilience and reduce mortality rates. This research not only advances scientific knowledge in the field of animal genetics but also offers practical solutions for improving the sustainability and economic viability of the sheep industry in New Zealand and beyond.

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Chapter 1

General Introduction

In New Zealand, the first three days of a lamb's life is critical as the lamb losses range from 5% to 25% during this period (Fisher & Mellor, 2002). Starvation and exposure account for up to 30% of the losses due to the cold (Everett- Hincks et al., 2007; Hinch & Brien, 2014). The number of losses is significantly affected by the weather conditions. The sheep industry in New Zealand and Australia is an outdoor pastoral system where newborn lambs are exposed to wet and cold conditions during the lambing period (Mellor & Stafford, 2004; Tait et al., 2015). Hypothermia is the main contributor to starvation/exposure, as it decreases the lamb's ability to milk-suckle (Alexander & Williams, 1966), which can in turn result in death from starvation as the lamb will rapidly use up their limited energy reserves (Dwyer & Lawrence, 2005). These losses have serious animal welfare implications (Dwyer, 2008) and significant economic consequences for sheep farmers, as it reduces the number of animals available for selection as replacements (Forrest et al., 2007).

Improving lamb survival has been the focus of many research studies due to the current outcomes. However, direct selection for lamb survival is inefficient due to it having a low heritability (Lopez-Villalobos & Garrick, 1999; Safari et al., 2005; Cloete et al., 2009; Everett-Hincks & Cullen, 2009; Boujenane et al., 2013). A potential alternative to improve lamb survival is to select for a trait that is moderately to strongly correlated with survival and has a higher heritability than lamb survival solely. Skin thickness is one such trait, being moderately to highly heritable (Slee et al., 1991; Janssens & Vandepitte, 2004; Tait et al., 2015; Soltani-Ghombavani et al., 2017) and positively correlated with lamb survival by improving thermoregulation (Soltani-Ghombavani, 2021, Graña Baumgartner, 2023). Additionally, it can be measured via ultrasound along with routinely-assessed production traits such as muscle and fat depth at around five to eight months of age in New Zealand (Beef and Lamb Genetics NZ, 2016).

In addition to the importance of reducing the heat losses from the skin surface, the thermoregulatory capacity of the new-born lamb plays a major role in survival by reducing sensitivity to cold exposure (Mellor & Stafford, 2004). Skin temperature is another trait that can be considered as an indirect selection trait for lamb survival rate. Skin temperature has a moderate to high heritability (Graña Baumgartner, 2023). Graña Baumgartner (2023), suggested that skin thickness and skin temperature have a favourable negative correlation of

-0.23. Utilising skin thickness and skin temperature together in a selection index has the potential to be valuable traits to consider for future breeding programs to indirectly help improve lamb survival.

Currently, there is little comprehensive information on both skin thickness and skin temperature as an indirect factor affecting lamb survival rate. It has been shown that they have potential to be important traits in this area (Graña Baumgartner, 2023). There is little research into the genetic analysis of these traits in sheep and the potential of using the traits in breeding programs in New Zealand. Furthermore, research on genomic regions and potential genes associated with these traits is limited. A genetic and genomic analysis of skin thickness and skin temperature can further our understanding of their influence on lamb survival. The use of genomic selection and finding genes related to both skin thickness and skin temperature would beneficial to help improve lamb survival.

Chapter 2

Literature Review

2.1 New-born lamb mortality and its impact on farm economy and animal welfare

Lamb survival rate is a major problem in the sheep production industry in New Zealand hill-country. New Zealand's hill country farming is usually made up of steep to rolling terrain, extensive grazing conditions, high rainfall, and low labour inputs per flock. Lamb losses tend to be in the range of 10 to 25% (Haughey, 1993; Everett-Hincks et al., 2007; Quintela, 2007). Lamb losses of up to 40% have been recorded on some New Zealand farms (Fisher, 2004; Morris & Kenyon, 2004), while farms in Australia have reported lamb losses of up to 30% (Jacobson et al., 2020). It is estimated that 74% of the mortalities occur during the first three days of a lamb's life (Jacobson et al., 2020). Hatcher et al. (2009) reported that in Australia, of the approximately 31% total deaths were in new-born lambs, 6% of deaths occurred either during the birth process or within the first 24 hours of life, 14% between days 1 and 7, 3% between days 7 and 30, and 8% between days 30 and 110.

To increase profit, farmers aim to increase the total weight of lambs being sold. The aim is to improve the number of lambs weaned and the weight of the lambs at weaning. A higher lambing percentage results in better efficiency in terms of kg of meat produced per kg of feed consumed or per ewe live weight (Morel & Kenyon, 2006; Mishra et al., 2007; Earle et al., 2017). A review conducted into lamb mortality by Kenyon et al. (2019), showed that triplet lambs had a higher mortality rate (32.5%) than single-born (10.5%) and twin-born (14.5%) lambs. The increased selection and proportion of multiple-born lambs contribute to high losses worldwide (Kerslake et al., 2005; Everett-Hincks et al., 2005a, 2005b) and in New Zealand (Table 2.1).

Table 2.1. Overall mortality rates for new-born lambs reported in New Zealand through time. A dot is displayed where no data is available.

Overall mortality rate (%)	Twin mortality rate (%)	Triplet mortality rate (%)	Reference
10 - 25	.	.	Dalton et al. (1980); Fisher & Mellor (2002); Hight & Jury (1970)
5 - 25	.	.	Kelly (1982)
15	.	.	Hinch et al. (1985)
18 - 24	.	.	McQueen (1986)
.	15 – 32[1]		Geenty (1997); McCorkindale (1999); Morris & Kenyon (2004); Sheath et al. (1999)
5 -25	.	.	Fisher (2004)
.	20	40	Kenyon et al. (2006)
.	.	56 (light bwt[2]) 40 (medium bwt[2]) 28 (heavy bwt[2])	Morel et al. (2008)
.	6	27	Kerslake et al. (2009)
11 -33	15	33	Kenyon et al. (2019)

[1] combined twin and triplet mortality rate %;
[2] bwt: birth weight

The sheep meat production industry in New Zealand is one of the most important revenue generators for the country. The exports from New Zealand contribute to 47% of the global trade (Morris, 2009). According to Beef + Lamb New Zealand (2024), the total lamb export revenues are forecasted to be $3.7 billion for the 2022-2023 season, which is a decrease of 5.7% from the 2021-2022 season. Lamb losses prior to the age of slaughter can have a significant negative impact on the farmers' profit. A study conducted in 2007 in New Zealand estimated that lamb losses cost New Zealand farmers over NZ$580 million annually (Everett-Hincks et al., 2007), while a study conducted in Australia in 2002 reported lamb losses to cost Australian farmers AU$250 million (Walker et al., 2002). Lamb losses decrease the selection potential by reducing the number of lambs to select from, which will further increase expenses (Forrest et al., 2007). When looking at these numbers, it indicates the importance of finding a solution to minimise the mortality of new-born lambs in New Zealand. The smallest improvement in lamb survival could increase the annual profit from sheep as stated by Everett-Hincks (2007). An improvement percentage of 2% in lamb survival would have an increase of individual farm returns by approximately NZ$3500 and boosted industry returns by NZ$47 million annually. Besides the economical perspective, increasing lamb survival rates would also improve the consumers animal welfare perceptions.

Consumers and society in general consider a high lamb mortality rate an animal welfare issue as well as an ethical issue (Mellor & Stafford, 2004; Ferguson et al., 2014; Dwyer et al., 2016). In a survey that consulted New Zealand farmers, the majority of the farmers believed that lamb survival was the most important of the research areas (Greer et al., 2015). Australian farmers have also expressed concern with the high mortality rates of new-born lambs and were worried about what consumers might perceive (Elliott et al., 2011). Research regarding ways to reduce new-born lamb losses is important to the sheep production industry as it will bring production, animal welfare and economic benefits to the industry.

2.2 Causes of new-born lamb mortality

Low lamb survival rates can be caused by many problems, but there are three complex interactions that cause complications to arise with the survival rate of new-born lambs. These interactions include climate conditions around lambing, rearing ability of the ewe and the lamb’s viability (McDonald, 1962; Eales et al., 1983; Alexander, 1984, 1988). Other factors such as genetics, ewe nutrition during pregnancy, trauma, infection and ewe and lamb behaviour could also contribute to lamb mortality (Haughey, 1993). Most lamb deaths are a result of starvation/exposure and dystocia (Kerslake et al., 2005; Everett- Hincks et al., 2007), with these conditions resulting in approximately 30% of the new-born lambs deaths. The remaining percentage of new-born lamb deaths can be caused by other problems such as post-natal infection, abnormalities and misadventure (Hight & Jury, 1970; Dalton et al., 1980).

2.2.1 Starvation and Exposure

Starvation and exposure are essentially a result of a lamb starving and being in a cold environment. The starvation usually occurs around the time of birth which mainly results from the effects of mismothering McCutcheon et al., 1981). This form of mortality can be classified as simple starvation which is the exhaustion of all body reserves, simple exposure, which is death by hypothermia, or a mix between both (McCutcheon et al., 1981). Starvation occurs when a new-born lamb has insufficient or total lack of colostrum and/or milk (Mellor & Stafford, 2004). This can also be caused by a new-born lamb's inability to suck adequately due to weakness, mismothering, or competition with litter mates (Alexander, 1984; Vermunt et al., 1995; Refshauge et al., 2016). The lack of food results in the lamb's brown adipose tissue being depleted due to being used up to keep warm in the cold environment (Ehrhardt. 2020). This results in hypothermia and then the death of the lamb. This cause of mortality is frequently seen in multiple born lambs due to their lighter birthweight, as starvation/exposure has a negative relationship with birthweight (Dalton et al., 1980; Le Floch et al., 2010). Lambs that are born heavier have a higher chance of survival in harsh conditions due to them having more energy reserves in the form of brown adipose tissue as well as having a better suckling drive for greater duration than lambs with lower birthweights (Hight & Jury, 1970).

There is a correlation of lamb mortality with weather conditions when the lambing period occurs. Severe weather conditions contribute to the rate of lamb mortality from starvation/exposure, especially when lambing outdoors (Slee, 1981; Mellor & Stafford, 2004; Tait et al., 2015). When a lamb is born into these conditions, going from the warm uterine environment to a harsh cold external one, it must immediately increase its rate of body heat production fifteen times more than what it was in utero to compensate for the heat loss during this transition (Alexander, 1962c). If this heat production increase is not met to the standards of the external environment, it can result in lamb death.

At birth, the body temperature of the lambs drops before it increases to a normal body temperature of 39-40°C within a few hours of birth. However, the initial drop can continue to further decrease and can result in death for some lambs (Alexander & McCance, 1958). When a lamb goes through a high rate of body heat loss, it results in the lamb going into a high metabolic rate. This high metabolic rate results in the rapid consumption of body reserves (Alexander, 1962a). Lambs are able to survive for three to five days without feeding while the breakdown of the energy stores occurs, in a warm environment; the survival time would be much less for a lamb in severe cold environments (McCutcheon et al., 1981). Lambs that have been starved are more likely to be more susceptible to hypothermia. It has been recorded that a lamb's suckling reflex decreases with the decrease in body temperature below 37°C (Alexander & Williams, 1966). This, in part, explains why most starvation/exposure deaths occur during the first three days of a new born lamb's life (McFarlane, 1961).

2.2.2 Dystocia

Dystocia is defined as a difficult birth due to an unassisted parturition or prolonged birthing requiring assistance (Jacobson et al., 2020). Dystocia is a significant cause of perinatal lamb mortality (Hight & Jury, 1970; Haughey, 1973; Holst et al., 2002). Between 5 and 50% of lamb losses within the first week of life are associated with difficult births (Everett-Hincks et al., 2007). This can be a result of foeto-pelvic disproportions (Haughey, 1993; Refshauge et al., 2016; Noakes et al., 2018) or mal-presentation either due to the position of the lamb in the birth canal or entanglement with siblings (Alexander, 1984; Ennen et al., 2013; Mostefai et al., 2019). Birthweight and dystocia have a negative relationship, where increase in birthweight of the new-born lamb increases the likelihood of dystocia (Everett-Hincks & Dodds, 2008; Geenty et al., 2014). When the birthweight of the lamb increases from an "ideal weight", there is a rise in death due to dystocia (Scales et al., 1986; Fogarty et al., 1992). In several reports, the death of heavy single-born lambs has been attributed to dystocia being the cause (Arthur, 1967; Tarbotton & Webby, 1999; Kerslake et al., 2005). Dystocia is more prevalent in lambs with high or low birthweights, high or low liveweights of ewes and small first-season ewes (Jacobson et al., 2020).

2.2.3 Starvation-Exposure / Dystocia

If a new-born lamb survives a traumatic birth, it is more susceptible to starvation/exposure when compared to lambs that were born without any difficulties (Eales et al., 1982). According to Dwyer and Morgan (2006), many new-born lamb deaths from starvation/exposure are first trigged by dystocia. Problematic and long labours can negatively impact the establishment of the ewe-lamb bond (Dutra et al., 2007) and can increase the likelihood of rejection behaviour of the dams (Dwyer et al., 2003). Traumatic births can also affect the ability of searching for the teat, suckling and maintenance of body temperature in new-born lambs (Eales et al., 1982;

Jacobson et al., 2020). In these cases, lambs have a very low chance of survival (Murphy & Lindsay, 1996), and if they do happen to survive, it is possible for these effects to impact negatively on their later life.

New-born lambs can be more susceptible to hypothermia due to impaired thermoregulation (Alexander et al., 1980), if they have suffered from hypoxia during a difficult birth as their heat production system tends to be depressed (Stott & Slee, 1987). New-born lambs that are impacted from the combination of starvation/exposure and dystocia would require human intervention to increase its chances of surviving (Jacobson et al., 2020).

2.2.4 Post-natal infections

Post-natal infections account for a small proportion of lamb deaths (Eales & Small, 1986; Refshauge et al., 2016), with an Australian study associating 4% of deaths to infections (Suter, 2010). New-born lambs do not get immunoglobulins before birth across the placenta so they absorb them by ingesting the colostrum during the first 24 to 48 hours of life (Ballabriga, 1980). A new-born lamb can be more susceptible to infections if they are unable to consume colostrum (Collins et al., 1985; Hodgson et al., 1999). Lambs that have succumbed to infections will show signs of fatigue, fever, listlessness, reduced social interaction, inappetence and discomfort. All the signs of sickness behaviour are linked to immunological responses to infection (Gregory, 1998). Lambs born outdoors are exposed to a more severe cold environment where starvation happens more frequently when compared to lambs that have been born indoors that are more susceptible to infection from being on used deep litter (Fisher and Mellor, 2002). Infections and cold exposure can cause the lambs to have reduced suckling ability, leaving the lamb unprotected from potential deadly infections with the lack of colostrum and immunoglobulins (Hinch and Brein, 2014).

2.3 Biological factors that have an impact on lamb survival

Lamb survival is a critical aspect of sheep farming, influencing both the economic viability of farms and the welfare of the animals. Several factors play a pivotal role in determining the survival rates of lambs. These factors include breed, sex, birth weight and body size, litter size/litter mate survival, lamb vigour, and ewe nutrition and age. The breed of the sheep and sex of the lamb can affect the potential survival of the lamb through genetics. The amount of lambs born can affect the starting conditions of the lamb. The ewe's health and nutrition during pregnancy directly impact lamb birth weight and vitality. Understanding and optimising these factors can lead to healthier lambs and more productive sheep farming operations.

2.3.1 Breed

It is known that lamb mortality can be affected by breed of sheep. Lamb losses from crossbred ewes are generally lower than the losses from pure breed sheep (Dalton et al., 1976; Fogarty et al., 2005). Some hill-based sheep breeds, (Cheviot, Scottish Blackface and Welsh, and non-domesticated sheep breeds, such as Borerary Blackface and Soay), have been seen to have higher survival rates than lowland sheep breeds, such as Border Leicester, Finnish Landrace, Oxford, Southdown, Tasmanian and Merino (Slee, 1981). A study conducted by Slee (1981) found that there were differences between different breeds for their ability to cope with a cold environment and Samson and Slee (1981) showed that lambs belonging to hill-based breeds had a higher ability to resist hypothermia.

Breed differences in lamb morality can also be due to the difference in ewe conformation, ewe milk supply and (Haughey et al., 1985), maternal behaviour. Merino ewes have been observed to have poor maternal characteristics, while in primiparous ewes are often associated with lamb desertion (Alexander et al., 1993). The difference between breeds in survival of lambs may be associated with birthweight, where heavier breeds have higher survival rates (Hinch & Brien, 2014), however, this can increase the risk of dystocia. Dalton (1980) showed that single-born lambs to Dorset-Romney and Cheviot ewes had higher rates of dystocia than single-born lambs to Coopworth and Perendale ewes. These results were also linked to differences in birthweight, birth coat and cold resistance. George (1976) stated that the occurrence of dystocia ranged from 4.1% in Merino lambs to 34% in Dorset lambs.

2.3.2 Sex

It is well known that male lambs have a higher rate of mortality to weaning when compared to female lambs (Dalton et al., 1980; Riggio et al., 2008; Matheson et al., 2012; McHugh et al., 2016), between 1-9% more (Hight & Jury, 1970; Johnson et al., 1982; Hatcher et al., 2009). A study done by Southey et al. (2001) showed that male lambs tend to have a 23% higher chance of mortality compared to female lambs within the first year of life. The difference in mortality rates between the sexes could be due to liveweight, as males are typically larger size at birth increasing birthing difficultly (Alexander et al., 1955; Hight & Jury, 1970; Jopson et al., 2000). Johnston et al. (1982) has reported that the survival variations to weaning of male and female lambs was higher in singletons, with 4.9% increased survival for females, than in multiples, with 2.3% increased survival for female lambs. It could be assumed that male lambs with greater liveweight at birth should have an increased effect on survival, as heavier lambs seem to have a greater resistance to cold exposure stress (McCutcheon et al., 1983). A study done by Stott and Slee (1987), showed that females tend to have a higher metabolic rate and a higher total body insulation than males regardless of their liveweight. Thus, the higher rates of mortality in male lambs may be due to a difference in thermoregulation.

2.3.3 Birth weight and body size

The birth weight of a lamb at birth is an important factor which can affect both single-born and multiple-born lamb survival (Hight & Jury, 1970; Hinch et al., 1985). This can be impacted by multiple factors including litter size, placental size, sex, diseases and dam nutrition during pregnancy (Alexander, 1984). Lamb mortality rate has a curvilinear relationship, an inverted U (Figure 2.1), with the birth weight of the lamb, where lamb mortality is maximum at birthweight extremes (Hight & Jury, 1970; Dalton et al., 1980; Holst et al., 2002; Geenty et al., 2014).

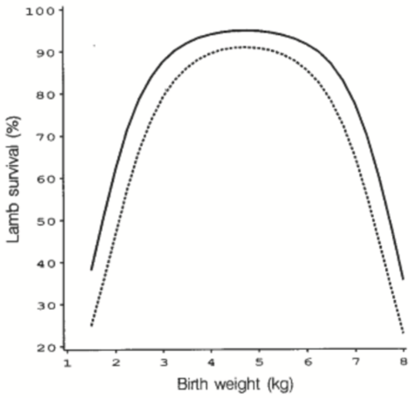

Figure 2.1. Lamb survival versus birth weight in Marshall Romney (solid line) and Romney (dotted line) sheep (reproduced from Knight et al., 1988).

Pettigrew et al. (2020) stated that the ideal birth weight for a large size breed was 6.5 kg. This would result in a survival rate between 86% and 91% when born to a mature ewe, compared to only a 60% survival rate if born to an ewe lamb. The best birthweight for a smaller bred ewe has been observed to be between 4.5 and 5.5 kg (Fogarty et al., 1992; Holst et al., 2002). This means that the optimal birthweight would be dependent on the breed (Hinch & Brien, 2014). For several New Zealand and Australian breeds of sheep, the greatest survival rate at birth have been recorded in lambs weighing between 3 and 5.5 kg (Dalton et al., 1980; Alexander, 1988; Morris et al., 2000; Sawalha et al., 2007). Previous studies show that there is a much larger variation in the survival of heavier and lighter lamb fetuses in triplets than in twins (Kenyon et al., 2007). These deviations from the average weight, specifically towards the lower weights, increase lamb losses (Petersson & Danell, 1985; Burfening & Carpio, 1993). These deaths are mostly due to starvation/exposure as a result of insufficient body reserves (Scales et al., 1986; Yapi et al., 1990).

Lighter lambs have a greater surface area to body weight ratio, which can negatively affect their thermoregulatory capability and survival (Sykes et al., 1976). In addition, smaller lambs have less insulation and cold resistance, lower body reserves and reduced ability to recover from hypothermia, compared to bigger lambs (Alexander, 1984; Stott & Slee, 1987). A deviation towards a bigger birth weight can lead to a higher mortality rate caused by dystocia or birth injury (Dalton et al., 1980; Fogarty et al., 1992; Nowak & Poindron, 2006). However, if the lambs are able to survive the birth process, chances of survival increases for these larger sized lambs (Morris et al., 2000).

2.3.4 Litter size and Litter mate survival

Several studies in the past have shown that lamb mortality usually increases with litter size (Petersson & Danell, 1985; Kenyon et al., 2006; Riggio et al., 2008; Hatcher et al., 2009), and can be in excess of 30% (Mellor & Stafford, 2004; Hinch & Brien, 2014). Nicoll et al. (1999) showed that mortality rates were lower for single-born lambs (9.9%) and increased with greater litter sizes (12.1% for twin-born and 26.4% for triplet-born lambs). Holst et al. (2002) also similarly observed losses of 11.1% for single-, 20.8% for twin- and 46.2% for triplet-born lambs, while Fowler (2007) also reported losses of 16.5% for singles and 31.5% for twin-born lambs. In triplet-born lambs, this higher mortality rate has been associated with the lower birthweights that this birth type has, and it has also been associated with much higher risks for starvation and exposure (Dwyer & Morgan, 2006).

There looks to be a relationship between the survival of multiple-born lambs and the fate of their littermates. A study by Hatcher et al. (2009) observed that survival rates from birth to seven days of age were higher for twin-born lambs when both lambs survived the birth process, 8% more than for those twin-born lambs who lost their littermate at birth. However, after seven days, the pattern of survival seemed to reverse. The lambs that had lost their littermate, prior to the seven days, had a higher survival rate to weaning than those with a surviving littermate. The authors suggested that this reverse situation was likely to occur due to competition for milk between lambs, as the energy demand increases prior to their intake of hard feeds. Similar situations have been previously seen when the dam's milk supply starts to decrease. Also, a particular lamb of a multiple-born litter could often be severely handicapped in its milk consumption compared to its littermates, which can increase the risk of its pre-weaning death (Hinch, 1989).

2.3.5 Lamb vigour

In the first hour or so of a lamb's life, the dam licks and grooms her new-borns as part of the formation of the exclusive olfactory memory. This will help them dry off and restrict her maternal care only to them, while rejecting the access of the udder to others (Keller et al., 2003; Nowak et al., 2007). Several reports have shown lamb vigour, being the lamb's behaviour during the establishment of the ewe-lamb bond, to be correlated with maternal efforts of bonding. This means that it is important that the lamb is not separated from its dam as this also has a marked relevant influence on the lamb's survival (Lindsay et al., 1990; Murphy & Lindsay, 1996). It is known that a poor bond between the ewe and the lamb is a major factor of lamb mortality. This is because it can cause hypothermia and starvation in the lamb which usually results in death on the day of birth (Dwyer et al., 2016). When the lamb's body temperature drops below 37°C, the sucking drive to be considerably depressed. If this happens, even a slight sign of hypothermia can pre-dispose lambs to starvation and death (Alexander & Williams, 1966). Good maternal behaviour, exhibited mostly by experienced ewes, can prevent this and facilitate lamb-sucking responses. However, the behaviour of the lamb will contribute, but if the ewe doesn't allow feeding ultimately determines the lambs survival chance (Dwyer et al., 2016).

2.3.6 Ewe nutrition and age

Maternal undernutrition can decrease lamb birthweight, negatively affect the ability of the lamb to thermoregulate (Dwyer et al., 2016), impact the mothering ability of the ewe and, the amount and quality of the milk and colostrum produced (Dwyer et al., 2003) and can also affect long term growth and productivity. Maternal undernutrition can also cause the ewe to have metabolic disorders, prolonged parturition, a shortened gestation period, decreased lamb vigour, decreased lamb's body lipid concentration and a late onset of lactation (McDonald, 1962; Hight & Jury, 1970; Haughey, 1993). Several studies state that foetal growth, lamb birth and weaning weight are less likely to be influenced by the nutrition of the ewe in early pregnancy than in the latter stages, except where the restriction is too severe (Symonds et al., 2007; Greenwood et al., 2010; Kenyon & Blair, 2014; Rooke et al., 2015; Dwyer et al., 2016). During the last six weeks of gestation, the unborn lamb gains 70% of their future birth weight and for that reason is susceptible to the low planes of the ewe's nutrition during this time (Hight & Jury, 1970). If maternal undernutrition reaches a severely low level in these final days of gestation, it can decrease foetal growth by 30-70% and, in some cases, can cause a cessation of the foetal growth (Mellor, 1983).

Younger ewes tend to give birth to lambs that have reduced lamb survival when compared with older ewes (Atkins, 1980; Knight et al., 1988; Riggio et al., 2008; Everett-Hincks et al., 2014). Lamb survival is usually based on dam's age and is highest for the 4-6 year old range (Knight et al., 1988; Lopez-Villalobos & Garrick, 1999), while ewes that are 2 years old or older than 6 years have the lowest lamb survival rates (Hatcher et al., 2009). It is suggested that this survival result could be due to mature ewes being more experienced giving birth to larger lambs, whereby increasing the rate of lamb survival (Dalton et al., 1980; Afolayan et al., 2007). Moreover, younger ewes have a higher risk of dystocia, since they are often smaller and have a disproportion between their size and their lamb's size (McMillan, 1983). This means that younger ewes tend to have a longer parturition and are more prone to requiring birthing assistance than mature ewes (Matheson et al., 2012). According to Fowler (2007), lamb survival was observed to be 6.1% lower in single-bearing maiden ewes, compared to single-bearing mature ewes.

2.4 Thermoregulation in new-born lambs

Thermoregulation in newborn lambs is a crucial physiological process that enables them to maintain their body temperature within a narrow, optimal range despite variations in environmental temperatures. Effective thermoregulation is vital for lamb survival, particularly in the first few hours and days after birth when they are most vulnerable to hypothermia. Newborn lambs have limited energy reserves and insulating fat, making them highly susceptible to cold stress. Their ability to generate heat through mechanisms such as shivering and non-shivering thermogenesis is essential for their survival. Understanding and supporting thermoregulatory processes in newborn lambs can greatly enhance their health, growth, and overall survival rates.

2.4.1 Thermoregulation / Heat production activation

When a lamb is born, it will go from a warm uterine environment to a cold external environment. This means that the neonatal lamb is required to increase its body heat production fifteen times higher than the foetal level to prevent heat loss (Dawes & Mott, 1959; Alexander, 1962c). If rectal temperature drops below 37°C, teat seeking ability is reduced in a new-born lamb and their metabolic rate capability starts to decrease (Alexander, 1962c; Alexander & Williams, 1968). The deep body temperature can decline resulting in hypothermia if the heat loss exceeds the lamb's highest sustainable metabolic rate or if the lamb fails to regulate its body temperature. If the body temperature of the lamb drops below 30°C, it can result in death (Alexander & McCance, 1958) thus, the ability of heat production in new born lambs is essential for survival.

Brown adipose tissue (BAT) located in lambs, helps with the ability to thermoregulate (Basse et al., 2015). BAT accounts for 60% of generated heat (Liang & Ward, 2006), and is the principal source of non-shivering thermogenesis. It is also crucial for the metabolic adaptation to low environmental temperature in the new-born lamb (Alexander & Williams, 1968; Symonds, 2013). Under cold exposure, the hypothalamic–pituitary axis releases catecholamines, such as norepinephrine. This in turn activates β-adrenergic receptors expressed on the surface of brown adipocytes (Contreras et al., 2015; Jiao et al., 2021). When these receptors are active, they can activate lipolysis and further release fatty acids in BAT (Forrest et al., 2007; Kurylowicz et al., 2015; Plush et al., 2016), which are the substrate for uncoupled oxidation and thus thermogenesis through uncoupling protein 1 (UCP1) (Cannon & Nedergaard, 2004). The key thermogenic factor UCP1, Thermogenin, is a BAT-specific transport protein of the inner mitochondrial membrane which helps by aiding the thermogenic ability of BAT (Ridley et al., 1986; Zingaretti et al., 2009; Whittle et al., 2011). These processes and inductions start to decline within a matter of days after birth in the new-born lamb (Finn et al., 1998; Shimizu et al., 2014).

2.5 Lamb body surface physiology and its relation to survival

Lamb body surface physiology plays a critical role in their survival, particularly in the early stages of life. The body surface of a lamb, including its skin and fleece, is integral to thermoregulation, protecting against heat loss and environmental stressors. Newborn lambs have a high surface area-to-body weight ratio, making them susceptible to rapid heat loss. The development and condition of their fleece, skin thickness, and subcutaneous fat all influence their ability to retain body heat. Effective thermoregulation is essential for maintaining body temperature, which is crucial for metabolic processes and overall health. Lambs with a well-developed fleece and adequate fat reserves are better equipped to withstand cold conditions, reducing the risk of hypothermia and increasing their chances of survival. Understanding the physiological characteristics of lamb body surfaces can help in implementing better management practices to enhance lamb survival rates.

2.5.1 Impact of coat type and depth on lamb survival

Numerous studies have demonstrated the significant influence of birth-coat type on lamb survival rate (Purser & Karam, 1967; Obst & Evans, 1970; Hatcher et al., 2009; Brien et al., 2010). In serve cold conditions, lambs with fine coats, such as Merino lambs, have exhibited lower survival rates when compared with lambs that have hairy coats (Cloete et al., 2009). However, these survival differences are reduced when the lambs are experiencing milder conditions (McCutcheon et al., 1981). Allain et al. (2014) posited that the main factor affecting lamb survival with respect to birth-coat types is the variation in coat surface temperature at birth. It was observed that the coat surface temperature of new-born lambs with long hair coats was 5.1°C lower than that of lambs with short, woolly coats. This suggests that lambs that have a longer coat tended to have better insulation with less heat loss into the environment.

Earlier studies have observed the impact of the type of birth coat on the ability of a new-born lamb’s ability to regulate their body temperature. Alexander (1962c) estimated that in still air, external insulation (coat and air) of fine-coated Merino lambs were 60% better then lambs with hairy coats. When the lambs were exposed to high windspeeds, the estimated external insulation of each coat type was halved. Similarly, Slee (1978) observed a correlation between the breed differences in mean birth coat depth and ability of lambs to regulate body temperature in severe weather conditions. These observations suggest that deeper coats experienced a greater reduction in depth when wet compared to finer coats, although the insulative value of a unit depth of coat remained constant when wet. This means, that having a wet coat can reduce its insulative value, which increases the rate of heat loss through the surface evaporation (McCutcheon et al., 1981).

Upon birth, a lamb's fleece is coated with amniotic fluid, rendering its thermal insulation ability ineffective soon after delivery (Hatcher et al., 2009). McDonald (1962) showed that a new-born lamb's birth-coat can retain up to 400 mL of foetal fluids. The dam can aid in drying the new-born lamb's coat through licking (Dwyer, 2008). Despite this, over half of new-born lambs remain wet or damp 90 minutes post-birth, with most lambs not being completely dry for at least three hours (Alexander & McCance, 1958). Achieving complete dryness involves evaporation, a process that absorbs heat from the lamb as it occurs (Alexander, 1962b). An estimated 100 kcal are required in order to evaporate 200 mL of fluid from the new-born lamb's coat (Moulk, 1954). This means that the coat type of the new-born lamb has an impact on thermoregulation in two ways; the insulation it provides (e.g., lambs with hairier birth coats conserve more energy than those with fine or medium coats) (Alexander, 1961, 1962a), and the amount of heat that is required to dry the fleece (McDonald, 1962). The variation in fleece depth may contribute to lamb survival as an advantage in unfavourable weather conditions, as greater fleece depth could enhance insulation capabilities (McCutcheon et al., 1981).

2.5.2 Skin physiology

The body's largest organ is the skin (Mlosek et al., 2021), compromising of 16% of the total body weight. Its primary function is to safeguard the body from environmental exposure and to provide external sensory awareness (Venus et al., 2010; Aspinall & Cappello, 2020). Additionally, the skin helps play an important role in the thermoregulation and immunological defence (Samuelson, 2007). The skin consists of two main layers, the epidermis and dermis, accompanied by the hypodermis which is a subcutaneous tissue that anchors the skin to the adjacent structures (Samuelson, 2007). Skin thickness can be classed as either thick or thin based on the thickness of the epidermis (Figure 2.2).

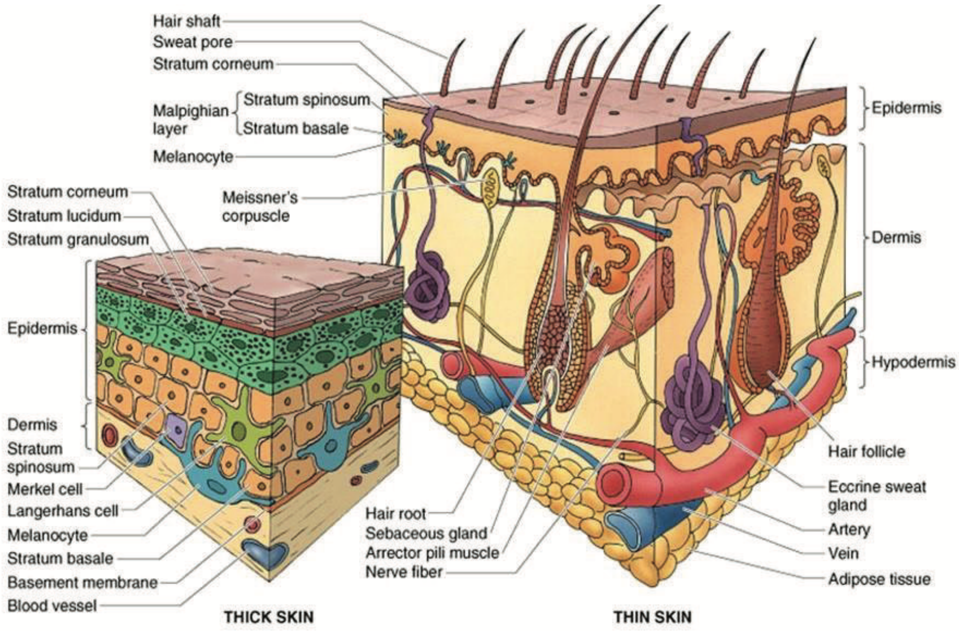

Figure 2.2. Schematic representation of the skin, comparing the structure of thick and thin skin (from Gartner & Hiatt, 2007).

The outermost layer of the skin, known as the epidermis, is a keratinized stratified squamous epithelium that acts primarily as a barrier to help prevent dehydration (Turton &Hooson, 1997). It has two main cell populations: keratinocytes that form the epithelium and non-keratinocytes such as macrophages, lymphocytes, and melanocytes (Samuelson, 2007). In response to an injury, the keratinocytes will produce cytokines that are associated with proinflammatory properties (Wilmer et al., 1994). The connection between the epidermis and the dermis occurs through the basement membrane, with thickness differentiating between regions that receive more abrasion compared to others (Samuelson, 2007).

Beneath the basement membrane and extending into the hypodermis, the dermis consists of dense irregular connective tissue with elastic, collagen and reticular fibres embedded in an amorphous ground substance (Dellman, 1993). The dermis is separated into the superficial papillary layer and the deep reticular layer with no clear line of demarcation between them (Dellman, 1993). The papillary layer consists of loose connective tissue that forms dermal papillae that strongly attach the epidermis and the dermis. Each dermal papilla has a capillary bed, which contributes to epidermal nutrition and regulating body temperature (Samuelson, 2007) by adjusting blood flow which means heat can either be dissipated or conserved (Melton & Swanson, 1996).

The reticular layer of the dermis features larger, more compact fibres compared to the papillary layer; in thick skinned animals, this layer of the skin appears to be very well-developed (Samuelson, 2007). Contained in this layer of skin are elements like glands (sebaceous and sweat), hair follicles and clusters of fat cells that can form in scatter clusters in the border of the reticular layer (Samuelson, 2007). Adipocytes within the dermis serve as an energy reserve and provide insulation to reduce heat loss (Aspinall & Cappello, 2020). In areas such as the around the kidneys, the adipose tissues can act as a protective layer (Aspinall & Cappello, 2020).

The subcutaneous tissue, or hypodermis, lies beneath the dermis and is comprised of loose connective tissue and fat cells (Dellman, 1993). The thickness of the hypodermis can vary with the physiological state of the animal, notably observed in animals that are storing subcutaneous fat for winter use (Turton & Hooson, 1997). This has also been observed in animals that are undergoing cyclical hair growth, where the hypodermis can be up to five times thicker during hair growth phases (Turton & Hooson, 1997).

2.5.3 Impact of skin thickness on lamb survival

In countries employing extensive outdoor production systems, the survival of lambs hinges on their ability to conserve heat and their thermoregulatory capabilities (Haughey, 1993; Mellor & Stafford, 2004). Consequently, lambs that are in such environments would benefit from enhanced insulative properties such as thicker skin, to help combat the cold exposure effectively (Tait et al., 2015). The skin of an animal plays an important role in body insulation by minimising heat losses from the surface of the body into the environment (Alexander, 1978). Many studies have demonstrated a positive and significant correlation between skin thickness and the cold resistance ability in new-born lambs, as well as an association with increased insulation (Samson & Slee, 1981; Stott & Slee, 1987; Slee et al., 1991). A recent study utilising calorimetry and infrared thermography on new-born Romney lambs exposed to cold and wet conditions concluded that lambs with thin skin experienced more significant heat loss through the skin, requiring increased heat production to maintain their body temperature (Soltani-Ghombavani, 2021). Therefore, the authors have suggested that a thicker skin could potentially enhance new-born lamb survival by increasing cold tolerance, a key component of lamb survival.

One key advantage of skin thickness is its moderate to high heritability as evidenced by various studies (Gregory, 1982; Slee et al., 1991; Janssens & Vandepitte, 2004; Tait et al., 2015; Soltani-Ghombavani et al., 2017). This observation holds particular importance because direct selection for increased lamb survival exhibits slow genetic gain, due to its low heritability estimate (Lopez-Villalobos & Garrick, 1999; Safari et al., 2005; Everett-Hincks & Cullen, 2009; Brien et al., 2010; Boujenane et al., 2013). Therefore, the feasibility of selecting for a thicker skin that could improve lamb survival becomes more apparent. The measurement of skin thickness through ultrasound at the farm can be routinely-measured at the same time as other economical traits, such as fat and muscle depth at around five to eight months of age (Tait et al., 2015). Ultimately, skin thickness could offer an alternative and cost-effective pathway for improving new-born lamb survival through selective breeding.

2.6 Methodologies for thermoregulation and lamb survival research

Research methodologies for studying thermoregulation and lamb survival encompass a range of advanced techniques that provide detailed insights into physiological processes and genetic influences. These methodologies include ultrasonography which is a non-invasive imaging technique used to evaluate body composition and organ development in lambs. This method could be applied to measure skin thickness in lambs. Infrared Thermography is a technique that measures surface temperature distributions, allowing researchers to monitor heat loss and detect areas of poor thermoregulation in lambs. It provides real-time data on how well lambs can maintain their body temperature. Estimation of genetic parameters (heritability and correlations) by analysing genetic variances and covariances by researchers can identify traits linked to better thermoregulation and survival.

2.6.1 Ultrasonography: skin thickness

Ultrasonography is widely used for skin examination in both clinical and research settings due to its speed, user-friendly nature, reliability and non-invasiveness (Schmid- Wendtner & Dill-Müller, 2008; Liu et al., 2017; Meikle et al., 2022). High-frequency ultrasound has been used for skin diagnostic analysis as at this frequency it offers sufficient resolution and depth to distinctly identify various skin structures (Alfageme, 2013). This technique works by detecting reflected sound waves through tissues with inherently different acoustic characteristics (Zanna et al., 2012). For instance, echoes in the dermis are formed from the reflection of ultrasound waves at the outer limits between dermal components such as reticular or collagenous fibres, or sebaceous and sweat glands (Gniadecka & Quistorff, 1996). Therefore, the ultrasound screen displays regions of different echogenicity that corresponds to various histologic layers (Aspres et al., 2003).

Real time ultrasonography has been demonstrated to possess advantages and accuracy in skin measurements compared to older methods in sheep, such as skin-fold calipers (Gregory, 1982a; Slee et al., 1991; Williams & Thornberry, 1992). A significant benefit of ultrasound over the caliper techniques is that the wool does not need to be clipped off before the measurement which can help prevent potential skin injuries and helps preserve the animal’s commercial value (Brown et al., 2000; Teixeira et al., 2008). The portability of the ultrasound equipment has led to several countries around the world to start including ultrasound measurements into genetic programs for lamb carcass quality improvement (Stanford et al., 1998; Tait, 2016). This means that including skin thickness measurements into these routinely-assessed economical traits seems feasible. This addition could offer crucial phenotypic data for ram breeders to help enhance genetic improvement programmes focused on cold tolerance and lamb survival.

2.6.2 Infrared thermography: skin surface temperature

Infrared thermography (IRT) stands out as a fast, passive, non-contact technique for generating a visual thermal profile (Vadlejch et al., 2010), and simultaneously can monitor larger areas (Lahiri et al., 2012). Utilising thermal cameras, IRT can capture the infrared radiation emitted by the study surface and transforms it into electrical signals, creating a thermal image that can help show the distribution of superficial body temperature (Speakman & Ward, 1998). An advantage of IRT is that it is completely non-invasive, which enables remote temperature readings without the influence of probes or human manipulation that might alter the temperature variation of the surface (Ludwig et al., 2014; Loyau et al., 2016). For these reasons, IRT has been proven to be a valuable tool in veterinary and animal research to detect body surface fluctuations (Stewart et al., 2005; McCafferty, 2007; Ferreira et al., 2011; Loyau et al., 2016). Infrared thermography has been extensively used in thermoregulation studies for these reasons (Jones, 1998; Bouzida et al., 2009).

Previous studies have applied IRT to assess radiated heat loss from the skin surface of new-born lambs while they were exposed to cold temperatures (McCoard et al., 2014; Soltani-Ghombavani et al., 2021) (Figure 2.3). McCoard et al. (2014) observed a rapid drop in skin surface temperature (from 35°C to 20°C) of lambs following birth, establishing that IRT as a useful method for measuring heat loss in new-born lambs. By taking advantage of measuring the skin temperature of lambs using IRT could create a sheep trait that can be utilised to indicate heat loss to the environment. If future studies are able to validate the fact that lower measured surface heat corresponds to better insulation, this trait could serve as an indirect indicator for lamb survival due to its significance in assessing cold tolerance.

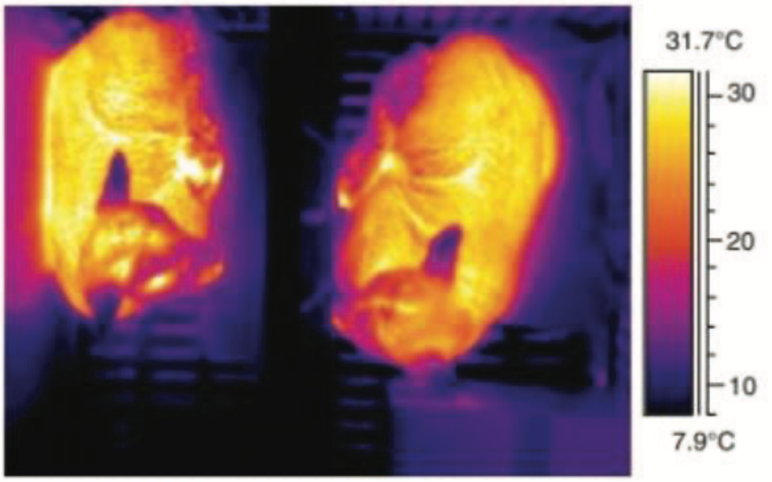

Figure 2.3. Thermal image of a pair of twin lambs (from McCoard et al., 2014)

2.6.3 Genetic parameters: skin selection for breeding programs

To utilize skin measurements, such as thickness through ultrasound or temperature through infrared thermography, into breeding selection, it is essential to assess the heritability of these phenotypes. Heritability is defined as the proportion of phenotypic variation attributed to additive genetic variation (Falconer & Mackay, 1996). Heritability estimates allow farmers and breeders guidance in determining the reliability of an animal’s phenotypic performance when selecting parents for the next generation (Cassell, 2009). Heritability estimates below 0.20 are considered low, those between 0.21 and 0.40 are moderate, and values that exceed 0.40 are high (Bailey, 2014).

There are limited studies on the genetic parameters of skin traits, particularly skin thickness, and far less studies have explored these traits to enhance lamb survival through improved cold tolerance (Tait et al., 2015; Soltani- Ghombavani et al., 2017). Studies that map skin temperature as a potential trait for improved lamb survival are also scare. Soltani-Ghombavani et al. (2017) looked at the genetic association of skin thickness with lamb survival. Using ASReml, animal models were applied to estimate genetic parameters. The heritability of skin thickness in Romney sheep was 0.26, which is a moderate heritability. The study suggested that skin thickness could be considered as a proxy trait for the selection of improved lamb survival. However, they also acknowledge more work was required to improve the estimates of heritability and genetic correlations between skin thickness and lamb survival.

Graña Baumgartner (2023) also looked at genetic improvement of new-born lamb survival through skin thickness and looked at skin temperature. The study looked at four breeds of sheep, FocusPrime, Highlander, Texel and Romney. It resulted in the heritabilities for skin thickness being mostly moderate (above 0.2) except for the Highlander breed being slightly lower. Skin temperature had relatively low heritabilities for all breeds expect for FocusPrime sheep that had a high heritability in FocusPrime sheep.

Tait et al. (2015) investigated skin thickness along with fat depth and eye muscle depth of sires and the correlation of these traits with related estimated breeding values (eBV) including lamb survival eBV of the progeny. Heritability for skin thickness in this study was 0.21 in Terminal Romneys. They also found that skin thickness was positively correlated with the progeny survival eBV. The results showed skin thickness had promise as an indirect selection criteria for improved lamb survival.

Table 2.2. Means and heritability estimates for skin thickness and skin temperature from previous research.

A dot is displayed where no records were available for the trait.

Trait	Mean	Heritability	Reference
Skin thickness (mm)	4.28	0.35	Slee et al. (1991)
	3.00	0.21	Tait et al. (2015)
	3.05	.	Jopson et al. (2000)
	2.92	0.26	Soltani-Ghombavani et al. (2017)
	.	0.22	Janssens & Vandepitte (2004)
	.	0.25 - 0.60	Gregory (1982a)
	.	0.28	Graña Baumgartner. (2023)
Skin temperature (˚C)	39.13	0.19	Morris et al. (1989)
	39.00	0.17	Dikmen et al. (2012)
	.	0.15	Graña Baumgartner. (2023)

2.7 Single nucleotide polymorphism genotypes

Single nucleotide polymorphisms (SNPs) are the primary source of variation in the genome. The SNPs serve as molecular markers for association with economically important traits in livestock. The ability to accurately and rapidly genotype SNP markers associated with traits of importance, while being cost effective, is an important component in genetically selecting animals for breeding (Niciura et al., 2018).

The development of SNP chips for livestock animals have significantly enhanced the ability to perform both genome wide association studies (GWAS) and genomic prediction. Genome wide association studies relies on the detection of the association between assayed SNPs and surrounding quantitative trait loci (QTL). The completion of GWAS and genomic prediction is influenced by the extent of linkage disequilibrium (LD) that persists between markers and the density of available markers to detect it (Kijas, et. al. 2014).

Following the developmental success of the bovine genome and bovine SNP Chips, the sequencing of the sheep genome and design of the first Ovine SNP chip was developed by the International Sheep Genomics Consortium (ISGC) (Oddy et al., 2007). Approximately, 60,000

SNPs evenly spaced across the genome were chosen for the Ovine SNP50 BeadChip, the final effective number of useful SNPs were 49,034 (Kijas, 2010). A high density SNP chip consisting of approximately 600,000 SNPs, with an average genomic spacing of 5_kb (kilobase pairs), was commissioned by FarmIQ^{TM} (joint New Zealand government and industry Primary Growth Partnership) and developed in conjunction with the ISGC and Illumina (FarmIQ, 2013; Kijas et al 2014). Further research has targeted small SNP chips that have been designed for specific purposes to be used in parentage testing and use in industry breeding programs. These breeding programs apply the use of SNP genotyping information from large numbers of individuals (Dalrymple et al., 2015).

2.8 Genome wide association studies

Genome-wide Association Studies have been an important attribute in mapping QTL associated with many genomic traits (Pickering. 2013). For most livestock species, 50,000 DNA markers can be genotyped in a single assay that has a low cost. During an analysis, the GWAS is able to utilize SNPs to detect loci across the genome that are associated with the trait of interest. The identification of these loci is dependent on the presence of LD. Traditional methods of GWAS involve the use of linkage studies, candidate gene studies, microarrays and fine mapping (Pickering. 2013). In a GWAS, each SNP is tested individually for the relevant association with the trait of interest. The underlying assumption is that the significant associations occur with the SNP because it is in LD with a causative mutation that can influence the trait, which indicates that the SNP is located near the mutation on the genome (Hayes and Goddard. 2010).

2.8.1 Techniques used in genome wide association studies

Marker-assisted selection (MAS) is a method where prediction of breeding values incorporates marker information and consists of a two-step procedure (Hayes and Goddard. 2010). The first step consists of identifying markers in LD with mutations affecting a trait of interest, known as QTL, as significant in the GWAS. The significant markers are then included into the prediction of the breeding value. The benefit of MAS compared to non-MAS methods is related to the proportion of genetic variance explained by QTL associated with the significant markers. The greater the genetic variance accounted for by these QTL, the more advantageous MAS will be for predicting breeding values (Hayes and Goddard. 2010). However, for complex traits (e.g., height), the proportion of significantly associated markers is low, as the variation of a trait is explained by a large number of QTL of lesser effect spread across the genome.

A different approach to GWAS involves using all genome-wide markers at the same time to predict breeding values, also known as genomic selection (Meuwissen et al., 2001). Marker-assisted selection in comparison, only uses the SNPs of significant in a GWAS. Genomic selection uses a genome-wide panel of dense markers that ensures that all QTL are likely to be in LD with at least one marker. Genomic selection can track the genetic variance for a trait by the marker as it does not require marker effects to surpass a significance threshold to predict the breeding value. The marker effects in genomic selection are not biased upward of multiple testing unlike those from GWAS (Beavis, 1998).

Genomic selection is used to improve New Zealand livestock through selective breeding using an animal's breeding values based on their genetic merit. By including genomic data into genetic selection, it has increased the accuracy of breeding values when compared to using best linear unbiased prediction (BLUP) methods based on relationships from known pedigrees. The additional accuracy gained from genotype information allows for the prediction of traits that are sex limited, measured late in life, and/or expensive to measure (Lee et al., 2021). It has been shown that in a study by Brito et al. (2017) that using the HD SNP chip had accuracies that support the feasibility of genomic selection for growth, carcass and meat quality in sheep. Relatively accurate molecular breeding values can be used to estimate various traits early in a lamb's life to determine traits that are difficult and expensive to measure (Brito et al., 2017). It has been recommended that by increasing reference populations with genetic selection can increase the accuracy and expected genetic gain in the future (Rupp et al., 2016).

2.9 SNP Imputation

The widespread adoption of genomic selection in dairy cattle followed the development of the Illumina SNP50 Genotyping beadchip. Subsequently, similar technologies were then released in sheep (Dodds et al., 2014) and beef cattle as reference datasets of genotyped animals with adequate size, along with SNP panels, became available. The next technological advancement was the use of lower density panels, which were more cost effective compared to higher density panels initially used. The lower density panels can be imputed to higher densities with high accuracy in cattle. In the New Zealand sheep production industry, imputation plays a crucial role for the implementation of genomic selection.

Imputation is a statistical approach that is used to infer SNP genotypes that are not directly obtained from a low-density panel. This is accomplished by using information from a reference group of animals that are genotyped with higher density panels (Ventura et al., 2014, Roberts et al., 2008, Su et al., 2008). Imputation is used to minimise costs of genotyping, but its accuracy can be influenced by many factors, which provides ample opportunity for the improvement and optimal implementation of this technology. In some animal populations, missing SNPs cannot be accurately inferred, and this is dependent on the structure of the reference population, being the group of animals genotyped with the higher density SNP, and the marker density of the reference and imputed populations. Improvements in imputation accuracy are closely associated with the level of relationship between the animals to be imputed and the reference population. It is also dependent on the number of animals in the reference population, the position of the SNPs on the chromosome, the density of the SNP panel used for the reference population and the breed composition (Ventura, et. al. 2014, Sargolzaei, et. al. 2014, Calus, et. al. 2014, Bolormaa, et. al. 2015).

2.10 Gap of knowledge and concluding insights

Lamb mortality in New Zealand is a common issue. There are four main causes of mortality being starvation and exposure, dystocia, starvation-exposure/dystocia, and post-natal infections. Factors such as breed, sex, birth weight and body size, litter size and litter mate survival, lamb vigour, and ewe nutrition and age, can influence lamb survival as well as skin thickness and skin temperature. Although many studies have been researched on lamb survival, there are only a few studies that have assessed skin thickness and skin temperature through ultrasound measurements (Tait et al., 2015; Soltani-Ghombavani et al., 2017; Graña

Baumgartner, A., 2023). The genetic parameters of the traits, from a lamb survival perspective, have been rarely studied (Graña Baumgartner, A., 2023).

The use of genomic technology, such as SNP chips, and methodologies, such as genome-wide association studies, imputation and genomic selection, has risen in use in animal breeding and has shown success in New Zealand with genomic prediction used in the national across-flock genetic evaluation managed by Beef + Lamb Genetics since 2018 (Lee et al, 2021). The identification of a gap in this area regarding GWAS for skin thickness and skin temperature presents opportunities for this to be studied. There has been no previous genome-wide association studies undertaken on skin thickness and skin temperature in sheep. This is an area that could be highly valuable for understanding the underlying genetic basis of traits related to thermoregulation and potentially improving lamb survival.

Therefore, the aim of this study were to analyse genetic parameters and perform a GWAS on skin thickness and skin temperature in FocusPrime sheep. The aim is to identify any SNPs of interest related to these traits and to see if there could be any underlying genes correlated with the traits that could potentially be used to breed indirectly for lamb survival in sheep.

Chapter 3

Materials and Methods

3.1 Animals and data collection

This study utilised a subset of data from a larger study on skin thickness and skin temperature in relation to lamb survival (Graña Baumgartner, 2023). To ensure the accuracy and reliability of results, skin thickness, skin temperature measurements and related animal data obtained from lambs born in 2017 on the FocusPrime flock on Pāmu (Landcorp Farming Ltd) Waikite Farm were selected (n=1236).

Skin thickness (ST) was measured on each lamb ultrasonically during August 2018 at approximately 11 months old. A commercial scanning operator was used to gauge these measurements by using a Mindray DP 50 ultrasound system (BCF Ultrasound Australasia, Auckland, New Zealand). It had a 40 mm probe at 3.5 MHz set on the left dorsal loin region of the lambs around the 12th rib. It was set up such that one mm of skin depth measured one cm on screen, and measurements were to a tenth of a mm (Graña Baumgartner, 2023).

Lambs had their skin temperature measured on the same day of skin thickness measurement. Skin temperature measurements (Stemp) were obtained through infrared thermography using an infrared camera (FLIR T650sc; Teledyne FLIR, Wilsonville, OR, USA), mounted on a tripod at a fixed distance (1.2 m at an angle of 50°). Prior to obtaining the infrared image, the wool at the site was parted to either side using a metal rod to capture an accurate recording of the exposed skin. Using FLIR Research IR Max software (Teledyne FLIR, Wilsonville, OR, USA), the exposed skin area on the infrared image for each lamb was delimited and utilized to provide an average of the lamb's skin temperature.

Skin thickness and skin temperature were measured on both ewe and ram lambs for the FocusPrime breed. Pedigree data and other phenotypes were obtained from Sheep Improvement Limited (http://www.sil.co.nz) and Focus Genetics (http://www.focusgenetics.com), and included: date of birth, sex (ewe/ram), flock, recording mob for each trait at the time of measurement, birth rank, rearing rank, dam age, dam and sire identities (Graña Baumgartner. 2023).

3.2 Data editing

Data editing was completed by Graña Baumgartner (2023), where lambs that had an unknown parentage in the pedigree dataset (n = 83) and those had insufficient records of birth rank-rearing rank (n = 35) were excluded from the final data set. Dam ages of 6-years-old or more were all considered as five-years-old (n = 254), due to relative low numbers. Similarly, lambs with a birth rank of four (n = 36) were considered as triplets and those with a rearing rank of four (n = 28) were classified as lambs with rearing rank of three, due to relatively low numbers. Outlier observations, values that were outside of the mean ± 4 SD range, were removed so all traits followed a normal distribution. After editing, the final dataset included 1236 FocusPrime lambs (born to 68 sires and 1501 dams).

3.3 Statistical analysis

Following data editing, the mean and standard deviation for each trait were obtained using the MEANS procedure of SAS 9.4 (SAS Institute Inc. 2013, Cary, NC, USA). Using the same software, analyses of variance for each dependent trait was performed using the generalized linear model procedure. The linear model included the fixed effects of the contemporary group, which was define as the group of lambs that were born in the same flock (birth flock 3003 or birth flock 4588), sex (ewe/ram), birth rank/rearing rank (born as single, twin or triplet, and reared as single, twin or triplet, 8 levels), the age of the dam (one to five years old, 5 levels) and liveweight at 11 months of age. Least squares means and standard errors were estimated for each class of the fixed effects and were used for multiple mean comparisons using Fisher's least significant differences. Means were considered significantly different when $P < 0.05$.

3.4 Residual values for GWAS

The residual values used in the GWAS was calculated through SAS using the general linear model procedure of SAS 9.4 (SAS Institute Inc. 2013, Cary, NC, USA). The analysis was performed on 1099 animal records from 2017 and two birth flocks. The residuals were based on the results from the MEANS procedure analysis.

3.5 Genotypes and Imputation

A total of 157 rams were genotyped using the high-density (HD) Ovine Infinium SNP BeadChip (606,006 markers) and 4,047 ewes, including dams of the 2017-born FocusPrime lambs, were genotyped using the LD Ovine 15K SNP Illumina BeadChip (n= 15,000 markers) according to the manufacturer's protocol. Genotyping was performed on a variety of public and custom genotyping arrays and results deposited into the Beef + Lamb NZ genotyping database. Genotypes were extracted from this database and assembled into one of three SNP sets, corresponding to the three arrays (ISGC consortium OvineHD, OvineSNP50K, ISGC_SheepLD15K), using the public array that had the most SNPs but no more SNPs than the chip on which the animal was genotyped.

The genotype quality control (QC) was implemented using Plink v1.9 (Chang et al., 2015). For the HD SNP chip, SNPs that: i) did not have a position on the ovine genome assembly Oar_rambouillet_v1.0 (GenBank acc GCA_002742125.1) ,ii) had a call rate (CRSNPs) > 10% and iii) had a minimum allele frequency (MAF) > 1%, were excluded for further analysis. No individuals had a call rate (CRINDs) below 90%. After QC and filters, the final HD dataset consisted of 519,928 SNPs and 157 animals. In the case of the LD chip genotypes, SNPs that did not have a position on the Oar_rambouillet_v1.0 genome assembly and were not included in the HD chip were removed. A total of 11,305 SNPs were retained for the analysis. The CRSNPs and CRINDs filters were not applied to the LD SNP chip since missing genotypes were assigned during the imputation process.

From 4,047 animals genotyped with the LD SNP chip, 3,233 had recorded parentage information. Thus, a population and pedigree-based imputation was performed (Whalen and Hickey, 2020). Pedigree information was used to impute genotypes for individuals with genotyped parents, while population-level information was used to impute genotypes for animals with ungenotyped parents and founders/ungenotyped animals present in the pedigree file.

For the imputation of genotypes from 11,305 SNPs to 519,928 SNPs, the software AlphaImpute2 (Whalen and Hickey, 2020) was used with default parameters. Finally, 6,186 animals present in the pedigree file were imputed to 519,928 SNPs genotypes. For the following gwas, the final genotype dataset used was those genotypes (original and imputated) of the born 2017 FocusPrime lambs (n=1099).

3.6 Genome-wide association analysis

The GWAS used the genome-wide complex trait analysis and restricted maximum likelihood packages (Yang et al. 2011). The creation of a genomic relationship matrix (GRM) was produced to then perform the genetic analysis. The genetic analysis used in the genome-wide complex trait analysis (GCTA) is the mixed linear model (MLM) association analysis. For studying the association between genotyped SNPs and the traits of interest, the following MLM was used in the study:

$$y=a+bx+g+e$$

Where y is the residuals of the phenotypes, a is the mean term, b is the additive effect (fixed effect) of the candidate SNP to be tested for association, x is the SNP genotype indicator variable coded as 0, 1 or 2, g is the polygenic effect (random effect), i.e., the accumulated effect of all the SNPs (as captured by the GRM calculated using all SNPs) and e is the residual. For the ease of computation, the genetic variance, var(g), is estimated based on the null model i.e. $y = a + g + e$ and then fixed while testing for the association between each SNP and the trait.

3.6.1 Q-Q plots

The QQ plot is used to assess the number and magnitude of observed associations between genotyped SNPs and the trait under study, compared to the association statistics expected under the null hypothesis of no association (Uzzaman et al., 2018). In the QQ plot, the observed association statistics ($-\log_{10}(P)$ values) are ranked in order from smallest to largest on the y-axis and plotted against the expected values from a theoretical χ^2-distribution under the null hypothesis of no association on the x-axis of the QQ plot. The Q-Q plots were generated using the qqman package (Turner, 2018) in R Studio (R Core Team 2024). If the distributions are similar then the slope, lambda, should be equal to 1. Any deviations from the identity line suggest either that the assumed distribution is incorrect or that the sample contained values arising from another source, such as true association. Since the underlying assumption in GWAS is that the vast majority of assayed SNPs are not associated with the trait, strong deviations from the null suggest either a highly associated and excessively genotyped locus (i.e., an associated gene with many genotyped SNPs) or spurious associations. Conversely, very little deviation from the expected values can be attributed to cryptic relatedness in the study population, genotyping errors, or true association (Uzzaman et al., 2018).

3.6.2 Identifying relevant SNP markers

Manhattan plots (-log*10*(*P*)) represent the *P* values on the y-axis against genomic order by chromosome and position on the x-axis of a sheep genome map, Oar_rambouillet_v1.0. Due to local correlation of genetic variants, arising from infrequent genetic recombination, groups of significant p-values tend to appear high on the Manhattan plot. The Manhattan plots were generated using the qqman package (Turner, 2018) in R Studio (R Core Team 2024). The P values are multiplied by log*10* to account for multiple testing in genome-wide association studies (Chen et al., 2021).

The 5% Bonferroni correction line and the suggestive line are put onto the Manhattan plots as an indication of significant SNPs of interest. The 5% Bonferroni correction method is a conservative method that selects a threshold *P*-value in genome wide association studies as it assumes that every genetic variant that is being tested is independent of the rest (Kaler and Purcell, 2019). The Bonferroni correction is calculated by dividing 0.05 (5%) by the number of SNPs in the dataset. With a total number of 519,928 SNPS in the dataset, the Bonferroni correction is 9.62×10^{-8}. The suggestive line is a line that represents the suggestive significance level. This is calculated by dividing one by the number of SNPs in the data set. The suggestive line for this dataset is 1.92×10^{-6}.

3.7 Candidate genes and functional analysis

Ensembl Release 109, based on the *Ovis aries* (sheep) reference genome in Oar_rambouillet_v1.0 genome assembly (http://www.ensembl.org/index.html, accessed on x? April 2024), was used to identify potential candidate genes. Gene annotation boundaries were set at 100 kbp (kilo base pair) on either side of significantly associated SNPs (Yates et al. 2020) identified from the GWAS. This range was selected due to the density of the chip array. The biological functions of the associated candidate genes were reviewed using the Gene Ontology (GO) tool in Ensembl.

3.8 Estimation of variances, covariances and heritability

The variance, covariances and heritabilities for each trait, from the gwas results, was calculated through the statistical package restricted maximum likelihood (REML) analysis (Lee et al., 2012).

The genetic correlation (r_g) between skin thickness and skin temperature was estimated as follows:

$$r_g = \frac{\sigma_{a12}}{\sigma_{a1} \times \sigma_{a2}}$$

where σ_{a1} and σ_{a2} are genetic additive standard deviations for skin thickness and skin temperature, respectively.

The phenotypic variance (V_P) were estimated as follows:

$$V_P = V_G + V_E$$

where V_G is the genetic variance and V_E is the environmental variance.

The heritability (h^2) for each trait was calculated as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_p^2}$$

where σ_g^2 and σ_p^2 are the animal additive genetic and residual variances, respectively, and their sum is the phenotypical variance.

Chapter 4

Results

4.1 Descriptive statistics

The number of records, mean and standard deviation (SD) for both skin thickness (ST) and skin temperature (Stemp) by variables are presented in Table 4.1 and 4.2, respectively. When comparing ST in the birth flocks (bflk), 3003 had a higher mean than 4588 whereas for Stemp, birth flock 4588 had a higher mean than 3003. Ram lambs had a higher mean ST, when compared to ewe lambs. Regarding Stemp, ewe lambs had a higher mean temperature compared to ram lambs. When looking at effect of age of dam on ST, dams that are five years old had a higher mean compared to other aged dams. One year old dams had the lowest mean for ST. For Stemp, two-year-old dams had the highest mean whereas the lowest Stemp mean was found in dams at one year of age. For birth rank and rearing rank combined (br_rr), the highest ST was found in lambs that were born as triplets and reared as twins. The lowest mean for ST was found in lambs that were born and reared as singletons. The highest mean Stemp for birth rank and rearing rank combined was seen in lambs that were born and reared as triplets whereas the lowest mean for Stemp was found in lambs born as triplets and reared as singletons.

Table 4.1. Mean (mm) and standard deviation (SD) (mm) and number of records (N) for each trait of skin thickness in FocusPrime New Zealand sheep.

Trait	Factor	Level[1]	N	Mean	SD
ST	Flock	3003	802	3.674	0.568
		4588	425	3.494	0.537
ST	Sex	E	665	3.444	0.534
		R	562	3.810	0.533
ST	Age of dam	1	132	3.551	0.577
		2	409	3.609	0.533
		3	318	3.612	0.516
		4	207	3.558	0.573
		5	161	3.879	0.57
ST	br_rr	1_1	256	3.572	0.541
		2_1	147	3.694	0.621
		2_2	705	3.606	0.559
		3_1	9	3.563	0.464
		3_2	42	3.743	0.566
		3_3	68	3.568	0.569

[1] E: ewe lamb; R: ram lamb; br_rr: birth rank_ rearing rank.

Table 4.2. Mean (°C) and standard deviation (SD) (°C) and number of records (N) for each trait of skin temperature in FocusPrime New Zealand sheep.

Trait	Factor	Level[1]	N	Mean	SD
Stemp	Flock	3003	696	37.344	1.588
		4588	403	37.699	1.384
Stemp	Sex	E	599	37.981	1.332
		R	500	36.867	1.521
Stemp	Age of dam	1	117	37.362	1.492
		2	366	37.540	1.575
		3	288	37.493	1.474
		4	185	37.377	1.657
		5	143	37.489	1.345
Stemp	br_rr	1_1	222	37.509	1.55
		2_1	134	37.334	1.514
		2_2	639	37.474	1.515
		3_1	9	36.822	2.05
		3_2	38	37.190	1.74
		3_3	57	37.961	1.238

[1] E: ewe lamb; R: ram lamb; br_rr: birth rank_ rearing rank.

4.2 Effect of environmental factors on skin temperature and thickness

F- and *P*-values of factors affecting factors affecting skin temperature and thickness are presented in Table 4.3. For skin thickness, sex and liveweight at 11 months were the most significant factors affecting the trait, with *P* Value less than 0.05. The F value for these factors, sex and liveweight at 11 months suggest that they are more relevant than other factors to this study. Skin temperature was significantly affected by sex, age of dam, and liveweight at 11 months.

Least squares means (LSMean) for each level of factors affecting skin temperature and skin thickness are presented in Table 4.4 and Table 4.5 respectively. For skin thickness, flock 3003 had a higher LSMean when compared to flock 4588. This is the same for skin temperature when looking at flock as a factor. For skin thickness, ram lambs had a higher LSMean when compared to ewe lambs. This was the opposite for skin temperature when looking at sex as a factor as ewe lambs had a higher LSMean compared to ram lambs. For skin thickness, 5-year-old dams had a higher LSMean when compared to the other dam age levels. This was the same for skin temperature when looking at age of dam as a factor. For skin thickness, lambs with a birth rank rearing rank of 3_2 had a higher LSMean when compared to the other birth rank rearing rank levels. For skin temperature, lambs with a birth rank rearing rank of 3_1 had a higher LSMean when compared to other birth rank rearing rank levels.

Table 4.3. F- and *P*-values for factors affecting skin thickness and skin temperature in FocusPrime New Zealand sheep.

Factor	ST		Stemp	
	F Value	*P* Value	F Value	*P* Value
Flock	0.70	0.4045	1.69	0.1932
Sex	48.35	<0.0001	26.00	<0.0001
Age of dam	0.84	0.502	2.56	0.0371
Birth-rearing rank	1.46	0.176	1.11	0.3527
LWT at 11 months[1]	6.09	0.014	14.08	0.0002

[1] LWT: live weight.

Table 4.4. Least squares means and standard errors of skin thickness for sex, age of dam and combinations of birth-rearing ranks in FocusPrime New Zealand sheep.

Trait	Factor	Level[1]	N	LSMean	SE
ST	Flock	3003	802	3.644	0.034
		4588	425	3.596	0.432
ST	Sex	E	665	3.505	0.041
		R	562	3.735	0.041
ST	Age of dam	1	132	3.576	0.064
		2	409	3.630	0.042
		3	318	3.628	0.041
		4	207	3.546	0.046
		5	161	3.719	0.052
ST	br_rr	1_1	255	3.573	0.035
		2_1	147	3.669	0.045
		2_2	702	3.607	0.023
		3_1	9	3.463	0.177
		3_2	42	3.756	0.083
		3_3	67	3.643	0.068

[1] Bflk: birth flock; E: ewe lamb; R: ram lamb; br_rr: birth rank_ rearing rank.

Table 4.5. Least squares means and standard errors of skin temperature for sex, age of dam and combinations of birth-rearing ranks in FocusPrime New Zealand sheep.

Trait	Factor	Level[1]	N	LSMean	SE
Stemp	Flock	3003	696	37.441	0.097
		4588	403	37.356	0.120
Stemp	Sex	E	599	37.846	0.115
		R	500	36.952	0.116
Stemp	Age of dam	1	117	37.251	0.181
		2	366	37.471	0.117
		3	288	37.411	0.114
		4	185	37.329	0.129
		5	143	37.532	0.145
Stemp	br_rr	1_1	222	37.518	0.101
		2_1	134	37.272	0.128
		2_2	639	37.386	0.064
		3_1	9	36.855	0.477
		3_2	38	37.094	0.233
		3_3	57	37.799	0.196

[1] Bflk: birth flock; E: ewe lamb; R: ram lamb; br_rr: birth rank_ rearing rank.

4.3 Genome-wide association study

4.4.1 QQ plots

The quantile-quantile (Q-Q) plots graphically compare two probability distributions to determine normal distribution. By looking at the Q-Q plot for ST, in Figure 4.1, it can be seen that the majority of the dataset for ST is normally distributed with the a little skew below the reference line. The Q-Q plot in Figure 4.2 also shows that the majority of the dataset for Stemp is normal distributed with a light skew above the reference line. Lambda for each plot was calculated to assess the distributions of the data. Both lambda estimates for the slope for ST and Stemp were 0.9996 and 1.001 respectively. These are equal to one meaning the data is normally distributed.

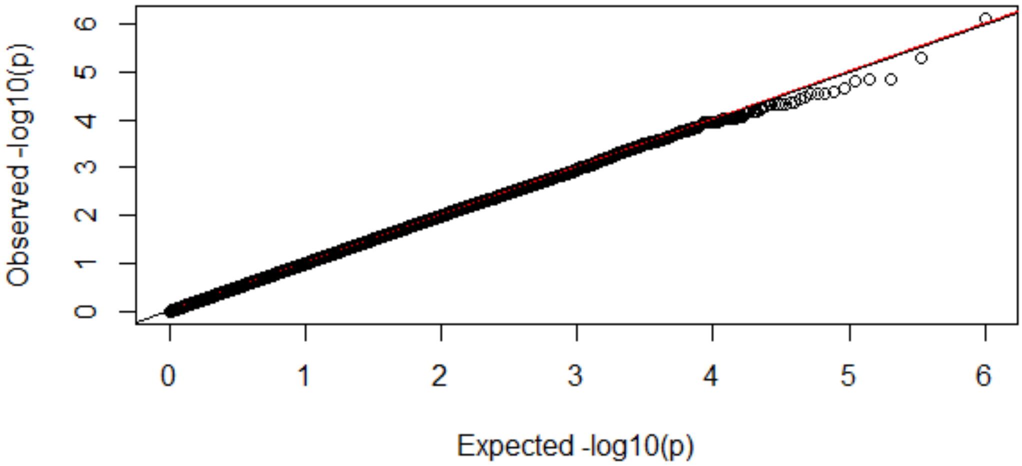

Figure 4.1. Q-Q plot for skin thickness -$\log_{10}$ (*P*), the 0-1 line is in red, and lambda is in black.

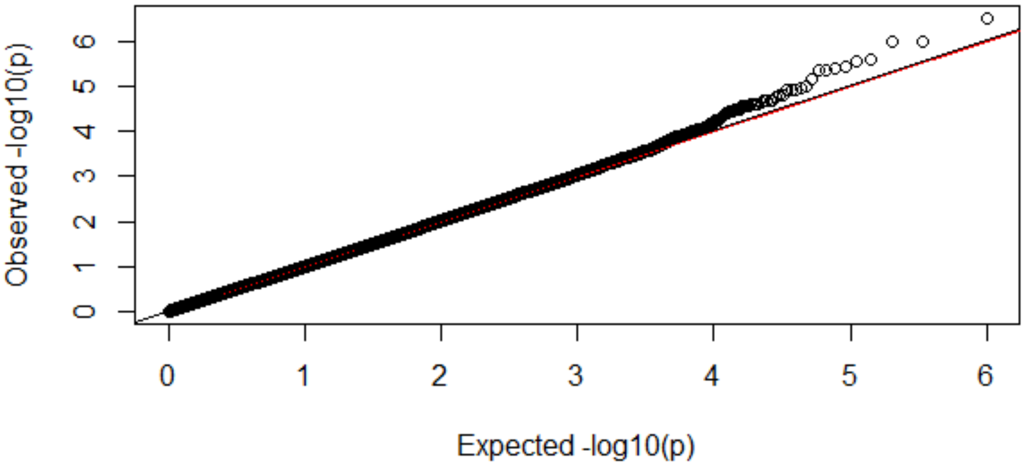

Figure 4.2. Q-Q plot for skin temperature -$\log_{10}$ (*P*), the 0-1 line is in red and lambda in black.

4.4.2 Significant SNPs for skin thickness and skin temperature

The profile of the P-values (in terms of -log(*P*)) of all the tested SNPs are shown in Figure 4.3 for ST and Figure 4.4 for Stemp. A single SNP on chromosome 2 was found to be significantly associated with ST (Figure 4.3), while three SNPs on chromosome 13 were significantly associated with Stemp (Figure 4.4). The details of the significant SNPs for both skin traits are shown in Table 4.6. Table 4.6 includes the significant SNPs, their chromosomal location and position, the raw *P*-value and genome-wide adjusted *P*-value, and the nearest known ovine genes. In total, three significant SNPs at the chromosome-wise level were identified for skin traits, one for ST (oar3_OAR2_36899998), and two for Stemp (oar3_OAR13_68477831 and oar3_OAR13_75344395). These significant SNPs achieved genome-wide significance levels of P<9.62x10^{-8}.

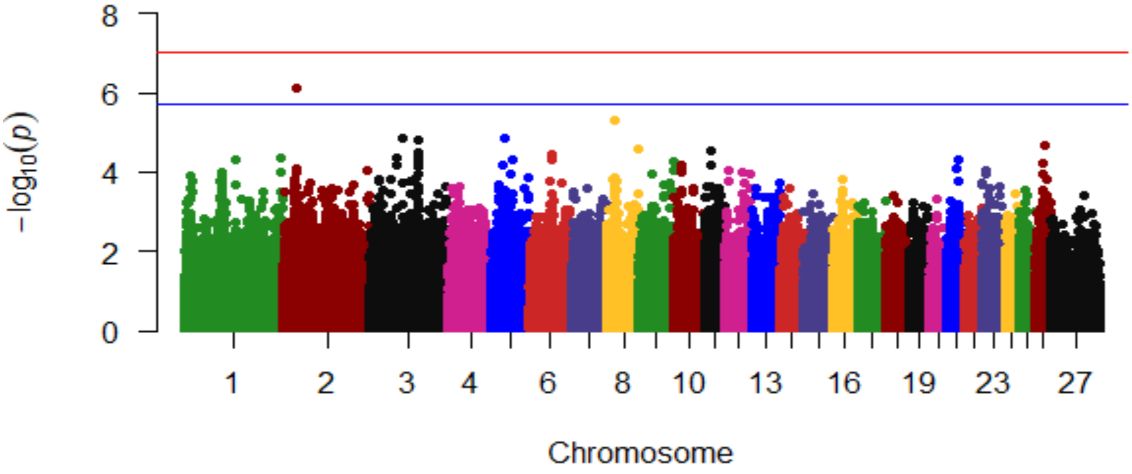

Figure 4.3. Plot of -log$_{10}$(*P*) values of SNPs for skin thickness, ordered on Oar_rambouillet _v1.0 map, P<1.92x10^{-6} (red), P<9.62x10^{-8}, (blue).

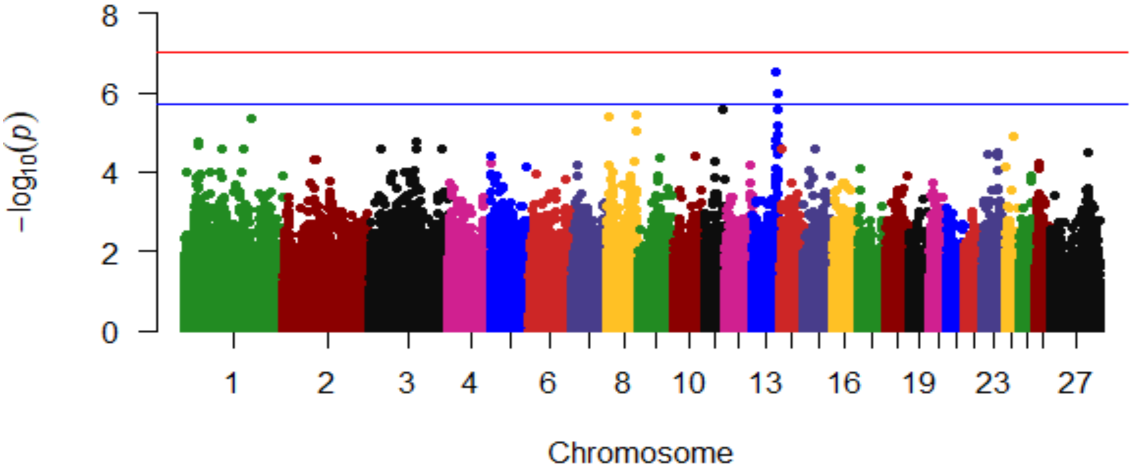

Figure 4.4. Plot of -log$_{10}$(*P*) values of SNPs for skin temperature, ordered on Oar_rambouillet _v1.0 map, P<1.92x10^{-6} (red), P<9.62x10^{-8}, (blue).

Table 4.6. Significant SNPs from the genome-wide associated study for skin thickness and skin temperature in FocusPrime New Zealand sheep.

Trait	Related SNPs	Chr[1]	Position (bp)	*P*-value	Adjusted *P*-value	Nearest gene distance (bp)
ST	oar3_OAR2_36899998	2	39090848	9.061E-07	6.04283	DPYSL2 within
	oar3_OAR13_68477831	13	71748805	3.06E-07	6.51399	PTPRT within
Stemp	oar3_OAR13_76481967	13	80289751	1.01E-06	5.99700	ENSOARG00020034342
	oar3_OAR13_75344395	13	79153388	9.695E-07	6.01343	NFATC2 +88340

[1] Chr: chromosome.

Ensemble and Ovine Ramb v2.0 (Ensemble, 2024) were used to identify genes within the region 100 kb either side of the significant SNP location. A further literature search and Online Mendelian Inheritance in Man (OMIM) was used to identify candidate genes. For ST, one gene with a role in growth cone collapse was identified. This was dihydropyrimidinase like 2 gene (DPYSL2) (Sherman, 1998). For Stemp, two gene were identified with roles in cell proliferation and cytosolic fraction of unstimulated T cells. These were protein tyrosine phosphatase receptor type T (PTPRT) (Baxter, 2004) and nuclear factor of activated T cells 2 (NFATC2) (McKusick, 1995). See table 4.7 for a description of these genes.

Table 4.7. Significant genes located from the genome-wide associated study for skin thickness and skin temperature in FocusPrime New Zealand sheep.

Gene Abbreviation	OMIM[1] Number	Description
Dihydropyrimidinase like 2 gene (DPYSL2)	602463	The DPYSL2 gene encodes a cytosolic phosphoprotein that can act as a mediator of growth cone collapse. It can modify axon number, length, and neuronal polarity
Protein tyrosine phosphatase receptor type T (PTPRT)	608712	Vital cellular functions, such as cell proliferation and signal transduction, are regulated by the balance between the activities of protein-tyrosine phosphatases
Nuclear factor of activated T cells 2 (NFATC2)	600490	It is a transcription complex is a multi-subunit transcription factor consisting of at least 3 DNA-binding components. It is present in the cytosolic fraction of unstimulated T cells.

[1] OMIM: Online Mendelian Inheritance in Man.

4.4 Heritabilities, variances and covariances

The heritability estimates for skin thickness and skin temperature are presented in Table 4.8. ST has a high heritability and Stemp has a moderate heritability, with both traits having low standard errors. Variance components (genomic, environmental, and phenotypic) for skin temperature were higher when compared to those for skin thickness. The genomic covariance between both skin thickness and skin temperature was negative, meaning that there was an inverse relationship between the variables. This was evident in the genetic correlation between the two traits.

Table 4.8. Heritability, variances, covariance and correlations (value ± SE) for skin thickness and skin temperature of FocusPrime New Zealand sheep[1].

Trait	h^2	$V_{(G)}$	$C_{(G)}$	$V_{(e)}$	V_p	r_g
Skin thickness	0.41 ± 0.08	0.12 ± 0.03	-0.16 ± 0.03	0.18 ± 0.02	0.30 ± 0.01	-0.56 ± 0.09
Skin temperature	0.35 ± 0.07	0.71 ± 0.17		1.33 ± 0.13	2.05 ± 0.10	

[1] h^2: heritability; $V_{(G)}$: genomic variance; $C_{(G)}$: genomic covariance; $V_{(e)}$: environmental variance; V_p: phenotypic variance; r_g: genetic correlation

Chapter 5

Discussion

5.1 Factors affecting skin thickness and skin temperature

Since heritability of lamb survival is near-zero (Everett-Hincks et al., 2005; Cloete et al., 2009), alternate strategies are required to genetically improve lamb survival. The use of indirect selection for a trait that has favourable genetic correlation with lamb survival would enable faster genetic progress. These traits could include skin thickness (ST) and skin temperature (Stemp). Selecting these traits, could be an achievable and cost-effective way to help increase lamb survival rate. The present study reports summary statistics and genomic analysis of ST and Stemp, and estimates of heritabilities and covariances in FocusPrime lambs.

Variation in sheep ST is not well documented (Brown et al., 2000), but where it is, ST LSmeans reported here are within the ranges reported in previous studies (Slee et al., 1991; Jopson et al., 2000; Tait et al., 2015; Graña Baumgartner, 2023). The slight variations in ST reported across studies may be due to differences in breed and the age at which measurements were taken. Additionally, the measurement techniques varied; some studies used skinfold calipers (Slee et al., 1991; Jopson et al., 2000), while others employed ultrasound machines (Tait et al., 2015; Soltani-Ghombavani et al., 2017). The accuracy of the ultrasound method can also differ. For example, Brown et al. (2000) noted a consistent difference in measurements depending on whether skinfold calipers or ultrasound were used. For instance, the ST of a 2-year-old Merino in summer could measure 1.74 mm with calipers but 2.56 mm with ultrasound. Furthermore, the time of year can influence ST measurements, as ST can vary with the seasons (Uchegbulam et al., 2022).

Skin thickness in lambs was influenced by the age of the dam. The lambs from younger dams (1-4 years) had less ST when compared to those from older dams (5 years). Knight et al. (1988) and Riggio et al. (2008) have both shown that older ewes have lambs with higher survival rates. These observations are based on the concept that mature ewes are more experienced dams and give birth to heavier/larger lambs, thus increasing new-born lamb survival (Dalton et al., 1980). Unfortunately, there is a lack of studies reporting a link between the age of a dam and their lambs' ST. This is an area that requires further research to allow for the non-genetic effect of dam age to be removed in the calculation of breeding values.

In the present study, Stemp and ST were measured as an indicator of heat loss and thermoregulation. Skin temperature was found to have a higher mean in ram lambs compared to ewe lambs. This is somewhat supported by Cloete et al. (2004) and Van Der Merwe et al. (2021), who found that rams had heavier skin weights and thicker leathers than ewes, though Jopson et al. (2000) reported no sex differences. Skin temperature had the highest LSmean in lambs who had a birth rank_rearing rank of 3_3. The lowest Stemp LSmean was found in lambs who had a birth rank_rearing rank of 3_1. Lambs with a dam that was 5-years-old had the highest Stemp compared to lambs with a dam of any other age. There is currently a lack of past studies reporting a link between Stemp with these factors. Therefore, the estimates of Stemp obtained require verification by addition studies to make appropriate comparisons and conclusions.

5.2 Selection for skin thickness and temperature and its potential impact on newborn lamb survival

Selecting for ST could be a viable method for indirectly improving lamb survival, given its heritability and correlation with survival rates (Tait et al., 2015; Soltani-Ghombavani et al., 2017, Graña Baumgartner, 2023). Combining the selection of ST with the selection for Stemp can help indirectly improve lamb survival rate. Skin temperature indicates heat loss is moderately heritable. Measurements of ST, via ultrasound scanning, around eight months of age can reliable reflect birth measurements (Soltani-Ghombavani et al., 2021). Consequently, selecting for ST in older lambs could enhance thermoregulation and survivability in newborns by improving heat insulation and reducing heat loss (Soltani-Ghombavani et al., 2021). Ultrasound scanning for ST and Stemp could be integrated into existing routines on New Zealand sheep breeding farms, where muscle depth is already measured at 5 to 8 months of age, with minimal additional cost and effort. As the study had skin measurements taken at 11 months of age, this could potentially impact the results if compared to birth and/or five to eight months of age. It is essential to understand how selection for these traits may impact production traits before incorporating them into breeding programs. Measurements should be taken at an earlier age to make further conclusions.

Skin thickness had moderate to highly heritable in the present study. This is comparable to those compiled from Janssens & Vandepitte (2004), Gregory (1982a), Tait et al. (2015), Soltani-Ghombavani et al. (2017), and Graña Baumgartner (2023) and Slee et al. (1991). Slight variations of heritability estimates can arise from differences in breeds, ages, measurement techniques, the time of year when measurements were taken, and the statistical models used, all of which can significantly affect heritability estimates.

The heritability for Stemp in this study was found as moderate to high heritability. The result obtained here were also similar to the estimates for rectal temperature in cattle, as reported by Morris et al. (1989) or Dikmen et al. (2012), and for Stemp in chickens (Loyau et al., 2016).

The correlation between ST and Stemp was negative. This means that increased selection for one trait, will decrease the other. Graña Baumgartner (2023) found that FocusPrime lambs had thinner skin and were in the middle to high range of ST when compared to other breeds. A higher ST indicates that more endogenous heat would remain within the body of the lamb and have less heat being lost to the environment. This therefore, maintains homeothermy in cold environments (Dwyer & Lawrence, 2005). This favourable correlation suggests that by genetically selecting for lambs with thick skin would decrease the level of heat that escapes out of the body surface into the environment. This would presumably have a positive effect on lamb survival and cold tolerance. However, there is a lack of data directly examining this association on lambs at birth.

Selection to increase cold tolerance is known to be positively associated genetically with traits that provide insulation, such as ST, fat depth, body weight, and wool (Stott & Slee, 1987). In other words, animals with thicker skin, larger body weight, deeper wool coats, and significant fat reserves are better suited to endure cold conditions and have a higher likelihood of survival under adverse conditions.

In addition to fat being a critical insulation trait, the combined effects of larger body weight and thicker skin at birth can significantly enhance cold tolerance (Plush et al., 2016). In this study, liveweight at 11 months of age was shown to be a significant factor that effects ST and Stemp in newborn lambs. Previous studies have also noted a positive correlation between birth weight and ST at birth. For instance, Soltani-Ghombavani et al. (2021) reported a 0.10 ± 0.02 mm increase in ST per kilogram increase in body weight, while Jopson et al. (2000) observed a 0.37 ± 0.06 mm increase. Thus, selecting for ST in new-born lambs would likely result in heavier body weights and thicker skin, enhancing body insulation and potentially improving lamb survival in adverse weather conditions during lambing. Genetic markers for ST would be valuable for trait selection.

5.3 Genomic selection for skin thickness and temperature and its potential impact on new-born lamb survival

In this study, there were three significant SNPs of interest for ST and Stemp. A SNP located on chromosome 2 at base position 39,090,848 was found to be significantly associated with ST. The dihydropyrimidinase like 2 gene (DPYSL2) was found within 200 kb window of the significant SNP (location is 2:39,055,180-39,171,255) and is a protein coding gene. It has currently been linked to cytoskeleton organization, nervous system development, regulation of neuron projection development and cell differentiation (Ensemble. 2024). DPYSL2 is known to play a role in neuronal development and polarity, and has a role in axon growth and guidance, neuronal growth cone collapse and cell migration (http://www.ensembl.org/index.html, accessed on May 2024). It is also involved in endocytosis and is a member of the collapson response mediator protein family (Pharos : Illuminating the Druggable Genome. n.d.). Cell migration is a vital process in the development and maintenance of multicellular organisms. This process allows for cells to move, change position and build or maintain tissues and organs. Cells can migrate as singular cells or multicell clusters (Moo-Young, 2019). Epidermal cell migration is the migration of the cells in the epidermis layer, the skin layer. This could potentially indicate the movement of cells to the epidermis layer to produce thicker skin in lambs.

Another function DPYSL2 is linked with is the central nervous system (CNS). The CNS has been directly (via efferent nerves or CNS derived mediators) or indirectly (via the adrenal glands or immune cells) connected to skin function. Sensory and autonomic (sympathetic) nerves influence a large range of physiological and pathophysiological functions within the skin. Physiological functions include body temperature and cell growth and differentiation. Direct stimulation by physical stimuli such as thermal can be detected in vitro and in vivo. It has been shown that mediators derived from sensory or autonomic nerves can play an important regulatory role in the skin where under physiological and pathophysiological conditions (Roosterman et al., 2006). There is potential that DPYSL2 gene might play a role in skin thickness in FocusPrime sheep.

Skin temperature had three significant SNP located on chromosome 13 at base position 71,748,805, base position 80,289,751 and base position 79,153,388. The gene protein tyrosine phosphatase receptor type T (PTPRT) was found within this region of the genome for the base position of 71,748,805. No significant gene was found at base position 80,289,751 of the genome. The gene nuclear factor of activated T cells 2 (NFATC2) was found within 88,340 mbps of the significant SNP.

The gene PTPRT (location is 13: 70,675,482-71,926,640) is a protein coding gene. It has been currently linked to protein dephosphorylation, homophilic cell adhesion via plasma membrane adhesion molecules, transmembrane receptor protein tyrosine kinase signalling pathway, dephosphorylation, negative regulation of cell migration, cellular response to interleukin-6 (IL6) and the negative regulation of receptor signalling pathway via STAT (Ensemble. 2024). Receptor protein tyrosine kinase are involved in the regulation of many cellular programs. The control of cell growth and differentiation are the most notable of the functions (Van Der Geer and Hunter, 1994). IL6 is a cytokine that is important for inducing the fever response during an infection. It has also been reported that it is able to uphold core body temperature during acute cold exposure. Egecioglu et al. (2018) looked at mice that were deficient for the IL6 receptor and mice that had the IL6 receptors. The mice were then exposed to long-term cold conditions to investigate if IL6 is important for core body temperature regulation. The results found that mice without IL6 receptors had a lower body core temperature when compared with mice that have the IL6 receptors. The results from this study suggested that IL6 was involved in not only the body temperature regulation during infection but also during long-term cold exposure, probably through mechanisms in the central nervous system (Egecioglu et al. 2018). Since the gene of PTPRT has been linked to the cellular response of IL6, this could indicate that the gene could help regulate the body temperature of newborn FocusPrime lambs.

The gene NFATC2 (location is 13: 79,241,728-79,397,510) is a protein coding gene. It has been currently linked to the positive regulation of gene expression, cell migration, the positive regulation of myoblast fusion and other biological process (Ensemble. 2024). The cell migration function could be similar to that found for the DPYSL2 gene. A myoblast is undifferentiated stem cells that contain precursors to skeletal muscle cells in the body. Myoblasts have many functions such as cellular development and differentiation (Liu et al., 2006). Myofiber formation in a sheep fetus usually begins at ~32 days of gestation and the full formation is usually completed by 80-120 days of gestation (Wei et al., 2014). Skeletal muscles help to maintain temperature homeostasis in the body by generating heat. Muscle contraction requires the use of energy and produces heat as a byproduct of metabolism. Myoblast fusion is an important step in the differentiation of muscle in most organisms and can involve tens of thousands of myoblasts. Because the myoblast formation happens during gestation, it allows for the muscle tissue to be able to regenerate in response to damage and disease. This could allow for the skeletal muscles to react in cold conditions in newborn lambs. It allows for the muscle to contract and produce heat in order to keep the lamb warm. Since this process dissipates heat into the body, it could therefore potentially decrease skin temperature and keep the body temperature of the lamb warm. Further research into this gene and its role would be needed to determine defined links to the traits.

5.4 Limitations of the study

There are a few limitations of this study that could be improved in further research. Using a larger sample size would benefit the research as it would give more power for detection of significant SNPs. Including other breeds and ages into this research would also help to identify any significant SNPs in New Zealand sheep population. Skin measurements were taken at 11 months of age but performing measurements at an earlier age i.e. five to eight months of age as being the recommended scanning protocol (B+L Genetics, 2016), would be beneficial and may help gather a more accurate insight to skin traits at birth. It was suggested by Soltani-Ghombavani et al, (2021) that skin thickness measured at eight months of age is a moderately reliable indicator of skin thickness at birth. Earlier measurement scanning could be further researched to see if is an indicator of skin thickness and temperature at birth. The validation of 11 months as a reliable measure of neonatal skin thickness could also be further researched.

When using imputed genotypes, there can be errors that arise due to the imputation method applied to impute these genotypes. These include the number of SNPs required to be imputed and the relatedness of the reference dataset to the set of animals to be imputed. In this study, sires of the born 2017 animals were present in the reference panel which would improve accuracy of imputation. However, having a medium density chip between the 15k SNP chip and the 600k SNP chip, such as the current 60K SNPchip provided by AgResearch, on a proportion of the animals would also improve imputation accuracy between the lowest and highest densities. These improvements could influence the GWAS results as the uncertain nature of the imputed genotypes may unavoidably bring biases to the downstream analysis (Zhang et al., 2022). The evaluation of different measures of genotype imputation correctness in the context of genomic prediction by Calus et al (2014) suggested that the correlation between imputed and true genotypes is the most useful and unbiased measure of imputation accuracy. It was also suggested that individual-specific imputation accuracies should be computed from genotypes that are centered and scaled.

5.5 Conclusions

In conclusion, this study has explored the genetic basis of skin traits in New Zealand FocusPrime sheep and their potential impact on lamb survival. The findings underscore the significant role that skin thickness (ST) and skin temperature (Stemp) play in the thermoregulatory capacity of lambs, which is crucial for their survival in the early days of life.

The heritability estimates for ST and Stemp suggest that these traits can be effectively selected for in breeding strategies aimed at improving lamb survival rates. Since both traits investigated in this study had an adequate amount of variation that is needed for genetic gain through selection. For ST, there would be sufficient phenotypic variation and moderate genetic control, implying that indirect selection for lamb survival through this trait could be achieved. It is suggested that it would be beneficial to also include Stemp as a trait itself, together with ST when selecting for lamb survival, since a moderate-high heritability estimate was recorded for

Stemp. Having both ST and Stemp as an indicator of heat loss from the body, can have the potential to be valuable traits to consider in future breeding programs to improve lamb survival. The negative correlation between ST and Stemp shows in increase in ST has a decrease in Stemp, meaning less body temperature is being lost to the environment making it a favourable response. The negative correlation further highlights the complexity of thermoregulatory mechanisms. This means the need for a balanced approach is required when selecting these traits in relation to other traits in the index.

There is some initial potential from the identification of some significant regions in the sheep genome for ST and Stemp. Identification of four potential significant genes that seem to have a role in ST and Stemp should be included through genetic selection methodologies in future studies. Studies regarding how thicker-skinned animals would endure hot conditions, and how those conditions might affect their performance would also be needed, so that no unfavourable effects were unintentionally selected for. By incorporating these genetic insights into breeding programs, it may be possible to enhance the resilience of lambs against harsh environmental conditions, thereby improving welfare and economic outcomes for sheep farmers.

Overall, the integration of genomic analysis into breeding strategies presents a promising avenue for reducing lamb mortality rates and ensuring the sustainability of the sheep industry in New Zealand. Future research should continue to refine these genetic and genomic associations and explore their practical implementation in a sheep dataset that reflects the diverse breed population present in New Zealand.

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