Genetic and genomic studies of production, composition, and processability characteristics of milk from dairy sheep

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science

at Massey University, Manawatū,

New Zealand

Ana Carolina Marshall

2024

Abstract

The main objective of this study was to perform genetic and genomic studies of sheep milk production, composition, and processability, particularly concerning cheese-making aptitude (milk coagulation properties) of sheep milk. The milk traits were investigated in a flock of 169 lactating ewes producing on a pasture-based system in Masterton, New Zealand. The total yield during the 2021-2022 season was 86.1 kg/ewe in 130 days of milking after the suckling period.

The effects of animal factors on the lactation curves, and on milk composition and processability (milk coagulation properties, individual laboratory cheese yield, and heat coagulation time) throughout the season were investigated and discussed. Stage of lactation significantly ($p < 0.05$) influenced processability. In late lactation, the rennet coagulation time was longer, the curd at 30 minutes after rennet addition was softer, and milk heat stability was lower. In addition, milk protein polymorphisms were shown to influence milk composition and protein composition. Particularly, heat stability was affected by β-lactoglobulin polymorphism. The milk processability traits were also significantly associated with protein composition.

The heritability estimates for milk production, composition, and processability traits ranged from 0.12 to 0.48. The genetic correlations obtained indicate that genetic improvement of this flock for higher yields of fat, and protein, and for lower somatic cell score, should indirectly improve milk coagulation traits in this flock. However, other traits such as milk pH, percentage traits (protein, casein, and lactose percentages), ratio of casein to protein, calcium, and ratio of casein to calcium were more strongly correlated with processability. The genome-wide association study performed on 149 dairy sheep genotyped with 50K SNPs Bead Chips, revealed a total of 87 SNPs and 55 candidate genes across *Ovis aries* autosomes 2, 3, 6, 16, 18, 20, 25, and 26. The genetic architecture of milk coagulation traits was similar to that of the ratio of casein to calcium, pH, lactose, and the ratio of casein to protein. The genetic correlations and identification of potential genes associated with the control of these milk traits provide valuable insights for the selection of superior dairy sheep in New Zealand. The findings of this thesis need to be validated with a systematic large-scale recording scheme before developing a selection index for dairy sheep populations in New Zealand.

Acknowledgements

I am profoundly grateful for life and the opportunities I have encountered and created. I appreciate the resilience and persistence I have developed, even during the most challenging moments, along with my faith in life and in something greater. I owe an immense debt of gratitude to my dear husband and family for their unwavering encouragement to pursue my dreams, no matter how challenging they may have seemed.

I am particularly thankful to Prof. Nicolas Lopez-Villalobos for believing in me and providing the exceptional guidance during my PhD studies and Masters. I also extend my gratitude to Prof. Warren McNabb from the Riddet Institute, leader of the NZ3M program, for allowing me to be part of this project. Your feedback during our supervisory meetings was instrumental in refining my thesis, as well as enhancing my communication and presentation skills. Thank you both for your immense support.

Also, my sincere thanks to Dr. Mike Weeks and Dr. Simon Loveday for their valuable contributions and constructive criticism. I am also grateful to the staff at the Riddet Institute—Terri, Michelle, and John—as well as to Massey University as a whole. Thank you all for your support.

I would like to express my heartfelt appreciation to Miles and Janet King from Kingsmeade Farm for their collaboration in this project and for permitting me to collect milk samples from their flock. Your contributions have been vital not only to my PhD research but also to the progress of the New Zealand dairy sheep industry. I admire your dedication.

I am especially grateful to my husband, Morgan Marshall, my partner in "crime," for always bringing positivity and calmness when I need it most. Thank you for your love and for standing by my side throughout this journey. I also want to thank my loving mother and best friend, Claudenyse Maria Reis Lacerda, for her unconditional love and emotional support in all my choices. I feel incredibly fortunate to have you as my mother. My aunt, Dr. Ana Cristyna Reis Lacerda, along with my mother, always motivated me to pursue education, a career, and independence. My grandmother, Evanyse Reis Lacerda, has lovingly cared for me throughout my life. You are my cheerleaders, my strength, and my pride. I have learned so much from these three remarkable women, and I consider myself lucky to have them as my family.

I am also thankful for my dog, Mello, who has been the best hugger and brings so much joy to my life. Without all of you, I would not have had confidence to follow my dreams, and for that, I am eternally grateful. Though we may not always be physically together due to the oceanic distance, we are always connected in heart and mind. I never feel truly lonely knowing I have your love and support.

Lastly, I would like to thank my PhD colleagues—Inthujaa, Dinesha, Vania, Elena, Zhang, Siqi, Marit, Ann, and Amber—for their support. I also appreciate the collaboration of Prof. Massimo De Marchi from the University of Padova and the guidance from Prof. Patrick Janssen at Massey University regarding the use of the Formagraph instrument. Thank you to everyone who has played a part in this journey.

Funding Acknowledgements

This thesis was part of a project funded by the Ministry of Business, Innovation, and Employment (MBIE) Endeavour Programme New Zealand Milks Mean More (NZ3M) Contract MAUX1803. Ana Carolina Marshall was supported by a Riddet Institute PhD Scholarship, and these are acknowledged.

Contents

List of Tables

List of Figures

List of Abbreviations

A30	Curd firmness at 30 minutes after rennet addition
Chr	Chromosome
CSNP	Casein percentage
CSNY	Casein yield
DM	Dry matter
DMLD	Deviation from median lambing date of the flock
FP	Fat percentage
FY	Fat yield
GO	Gene Ontology annotation
GWAS	Genome-wide association study
h^2	Heritability
HCT	Heat coagulation time
HPLC	High-performance liquid chromatography
ILCY	Individual laboratory cheese yield
K20	Time to reach curd firmness of 20 mm after start of coagulation
LP	Lactose percentage
LSM	Least squares means
LY	Lactose yield
MCP	Milk coagulation properties
ME	Metabolizable energy
MU	Milk urea
MY	Milk yield
NDF	Neutral detergent fibre
NEB	Negative energy balance
OAD	Once-a-day milking frequency
OMD	Organic dry matter digestibility
PCR	Polymerase chain reaction
PP	Protein percentage
PY	Protein yield
RCT	Rennet coagulation time after rennet addition
RP-HPLC	Reversed-phase high-performance liquid chromatography
SCC	Somatic cell count
SCS	Somatic cell score
SD	Standard deviation
SE	Standard error
SNP	Single nucleotide polymorphism
t	Repeatability
TAD	Twice-a-day milking frequency
α-LA	Alpha lactalbumin
α_{s1}-CN	Alpha s1 casein
α_{s1}-CN	Alpha $_{s}$2 casein

β-CN	Beta casein
β-LG	Beta lactoglobulin
κ-CN	Kappa casein

Chapter 1

General introduction

The New Zealand dairy sheep industry started in 1990 and drew valuable lessons from the well-established New Zealand dairy cow sector. However, research on milk production of dairy sheep within New Zealand's pasture-based farming systems remains limited. Furthermore, there is a notable gap in studies regarding the milk processability from individual sheep milk samples, especially within the New Zealand context. The composition and the physicochemical properties of sheep milk, which influence its processability, are determined by various factors, including genetics.

Dairy products from sheep milk are considered of high value, differentiated, and therefore are usually targeted towards health and gourmet food niche markets (Lees and Lees 2016). While sheep milk in New Zealand is primarily used for milk powder and infant formula exports, globally, its main application is in cheese manufacturing, due to its excellent coagulation properties compared to milk from other ruminant species (Park et al. 2007; Raynal-Ljutovac et al. 2008; Balthazar et al. 2017).

Understanding variations in milk coagulation properties among individual milk samples is valuable for identifying animals that produce milk with superior processing ability, such as for cheese-making. Also, it would help to understand the implications of animal selection on milk production and processability. There is potential to use genetics and genomic approaches for the selection of animals that are profitable for the farming businesses, producing high yields, but also that produce milk with the best technological properties. Previous studies have shown milk coagulation traits to vary across and within breeds of ruminant species.

Therefore, the general objective of this thesis was to understand the genetic and genomic bases of sheep milk production, composition, and processability characteristics, identifying chromosomal regions and potential genes associated with the genetic control of these traits. This information will provide insights for the genetic and genomic selection of superior animals for the New Zealand dairy sheep industry. The specific objectives of this thesis were the following:

- To evaluate the effect of animal factors (age, stage of lactation, coat colour or genetic variety, and litter size) on milk production (milk, fat, and protein yields), milk composition (contents of fat, protein, lactose, casein, calcium), processability characteristics of milk for cheesemaking (milk coagulation properties and individual laboratory cheese yield), with a secondary emphasis on

milk suitability for processing into liquid products like UHT milks (heat coagulation time).

- To evaluate the effect of protein polymorphisms on sheep milk processability traits.
- To estimate genetic parameters (heritability and genetic correlations) for the milk production, composition and processability traits.
- To perform genome-wide association studies for understanding of the genetic architecture governing the above-mentioned dairy traits.

This project is part of the New Zealand Milks Mean More (NZ3M) Endeavour Programme, which is funded by the New Zealand Ministry for Business, Innovation and Employment. The Programme aims to obtain a better mechanistic understanding of the natural variations in New Zealand bovine and non-bovine milk samples, including the structural and compositional properties of milk before processing.

Chapter 2

Literature review

This review informs on the overall current state of the New Zealand dairy sheep industry, along with a brief historical perspective on the development of this sector. It then presents the key intrinsic compositional and physicochemical properties of milk as well as animal factors that are known to impact processability. Finally, it summarizes the genetic parameters and genome-wide association studies available for dairy sheep traits, highlighting how genetic and genomic research offers significant opportunities to advance the industry and meet future demands.

2.1 The New Zealand dairy sheep industry in the world

The New Zealand dairy sheep industry, which began just over 30 years ago, is considered relatively new compared to the well-established dairy cow and meat sheep farming sectors in the country, as well as to long-established dairy sheep industries in other parts of the world, such as those countries in the Mediterranean, like France, Greece, Italy, and Spain (Peterson and Prichard 2015; Pulina et al. 2018). Although representing only 0.1 % of the world's sheep milk (Ministry of Primary Industries and Massey University. 2020), the New Zealand dairy sheep industry can potentially be worth more than $750 million in annual export receipts by 2035 (Scale up 2022). The highest average raw sheep milk production in the world occurs in Asia (45.2%), followed by Europe (30.5%), Africa (23.6%), and the Americas (0.9%). China is the largest producer of sheep milk, yielding approximately 1.2 million tons annually (FAO 2022).

Sheep milk is primarily used for cheese production in the Mediterranean basin, where it holds Protected Designation of Origin (PDO) status. Traditional cheese-making practices have been established in these countries for centuries (Carta et al. 2009; Pulina et al. 2018). Therefore, New Zealand's dairy sheep market strategy has focused on differentiated products that are also of high value such as infant formula, with its main importers being China and other Asian countries (Lees and Lees 2018). However, it has been recently noted that the New Zealand dairy sheep industry has been trying to reach other overseas markets, through the sale of other products such as cheese. For instance, Spring Sheep Milk Co.'s first commercial volumes of New Zealand-produced sheep milk cheeses are predicted to go into USA specialty stores at the end of 2024 (Radio New Zealand 2024a).

New Zealand's marketing strategy for its dairy sheep industry focuses on pasture-based production, emphasizing high-quality products, sustainable practices, and high animal welfare standards (Lees and Lees 2018). This approach offers a point of differentiation, as most sheep overseas are farmed indoors and offered total mixed rations. Griffiths (2015) developed a business plan for the sheep dairy industry, recommending not only marketing strategies but also collaborative efforts among producers to effectively market and sell overseas. This strategy would help establish consistent milk quality standards and maintain stable sheep milk prices for farmers. While many small individual dairy sheep farms still operate independently, the two larger companies, Spring Sheep Milk Co.and Maui Milk Limited, have scaled up. Spring Sheep, for example, now has 15 suppliers milking 15,500

ewes (Nick Hammond for Radio New Zealand 2024b), while Maui Milk has over 11,000 dairy ewes and 13 suppliers.

New Zealand has the potential to market fine sheep milk cheeses that reflect the country's unique environment, sheep breeds, high biosecurity standards, and commitment to sustainability and animal welfare, further enhancing its international reputation in the dairy industry. The existing artisanal knowledge from small farm businesses could serve as a valuable foundation for this initiative, blending traditional expertise with modern innovation. New Zealand artisan wines, for example, have successfully reached international markets.

Smaller commercial farms supply domestic markets with artisan cheeses, yogurt, and beverages, or they supply milk to other farms. Kingsmeade Farm (Masterton), which has been operating since 1996, manufactures artisan cheeses (Peterson and Prichard 2015, McCoard et al. 2023). Other small-scale farms include Fernglen (Riverdale, Wairarapa), Waimata Cheese (Gisborne), Charing Cross Sheep Dairy (Canterbury), Craggy Range Sheep Dairy (Havelock North). Other smaller farms supply milk to New Zealand’s two major sheep milk companies, Spring Sheep and Maui Milk (Ministry of Primary Industries and Massey University 2020).

The importance of milk composition is often reflected in payment systems (Sneddon et al. 2013). For example, in New Zealand dairy cow farming, the latest forecast predicts a payout of $7.75-$9.25 per kg of milk solids (Fonterra Co-operative Group Ltd 2024), which includes fat and protein, with penalties applied for excess volume due to processing costs. However, in the case of New Zealand sheep milk, payment is still based solely on volume, with no system yet in place to account for milk solids.

New Zealand's dairy sheep population saw rapid growth between 2015 and 2020, with an annual increase of approximately 12%. By June 2019, there were 18 commercial dairy sheep farms in the country, with a national flock of 12,345 ewes. However, during the 2023-2024 milk production season, many dairy sheep companies were affected by an imbalance between supply and demand for New Zealand sheep dairy products. For instance, Maui Milk decided to end its milking season earlier, in February (Radio New Zealand 2024c). Although the demand for infant formula has been established, particularly with China's slowing population growth, a new market opportunity has emerged in the pet industry, where sheep milk products are being sold at premium prices (Murray 2024).

More recently, the New Zealand dairy sheep sector has partnered with governmental bodies, research institutions, manufacturers, and producers to foster its growth into a more established industry. Examples of this include the Ministry of Business Innovation and Employment (MBIE) programme 'New Zealand Milks Mean More (NZ3M)' (supported by The Riddet Institute and its research and industry partners), the MBIE research programme 'Boosting exports of the emerging NZ dairy sheep industry' (supported by AgResearch and research partners, Spring Sheep Milk Co., and Horizon 3 programme), and the 'Sustainable Food and Fibre Futures' Ministry of Primary Industries (MPI) programme (McCoard et al. 2023). Key areas of research include on-farm production and environmental footprint, milk composition, processing, and functionality.

2.2 Dairy sheep farming in New Zealand

The temperate climate in New Zealand is considered a competitive advantage due to year-round pasture-based feeding options (Lees and Lees 2018; McCoard et al. 2023). Traditionally, New Zealand farming was characterized by being extensive, relying solely on pasture, with no housing or supplemental feeding, making it a low-cost system (Morris 2017). However, this model is changing, with greater use of supplemental feed and hybrid systems, where ewes might spend part of their time indoors, especially during the lambing period, along with intensification of grazing systems. This shift is particularly evident in larger commercial farms, which have been adopting advanced technologies to enhance productivity.

Overall, New Zealand dairy sheep farms still utilize a fully outdoor pasture-based grazing and management system (>75% forage) with up to 25% supplementary feed primarily in the milking parlor (McCoard et al. 2023). In 2020, most of NZ dairy sheep farms were on flat grasslands, of 31-40 hectares, with no housing, and operating only seasonally (Ministry of Primary Industries and Massey University 2020). It has been noted that more research is needed on the most effective way to feed dairy sheep in New Zealand farms, to meet metabolizable energy requirements, whilst considering the composition of the feeds available and the substitution effect when ewes are fed concentrates (Peterson 2017). In grazing systems animals are exposed to the weather/environment and the health problems that can be associated include facial eczema, ryegrass endophyte toxicosis, endoparasites, lameness, and nutritional stress (Kilgour et al. 2008).

Some farms in New Zealand, such as Kingsmeade Farm and Fernglen Farm, adopt a more traditional lamb weaning system, characterized by an exclusive lamb suckling period during the first four weeks after parturition, approximately, followed by a period of exclusive milk collection, as shown in Figure 2.1. Others use a mixed regime of lamb suckling and milk collection during the first month post-partum, which is possible to manage for highly productive ewes. Alternatively, the lambs are taken off their dams 48 hours post-partum and are reared artificially. The best choice of lamb feeding systems depends on various factors, such as the availability of skilled labor and infrastructure. Additionally, the presence of high-quality pasture is a crucial consideration (McCoard et al. 2023). The standard farm practice in New Zealand dairy cattle farming is to remove the calf from the cow around 24-48 hours after birth.

The temperate climate in New Zealand also means that most dairy sheep breeds will breed only seasonally as they are short-day breeders. Ewes start cycling in the seasonal transition from long to short days. In addition, they cycle according to feed availability, whereas dairy cows cycle all year round. Natural mating is more commonly used at commercial farms, as artificial insemination (AI) is more difficult in sheep compared to cows due to the anatomy of the reproductive tract of sheep. Therefore, AI in sheep is usually restricted to nucleus flocks, due to its surgical nature (laparoscopic). To assist with correct pedigree recording, parentage testing can be performed on lambs (Morris 2017). Because of natural mating, the mating and therefore the lambing periods in a dairy sheep farm are usually longer than the calving period in a dairy cow farm in New Zealand. Another consequence of the limited use of AI in sheep is the slower/limited dissemination of sire genetics and reduced genetic improvement through the sire line compared to cattle.

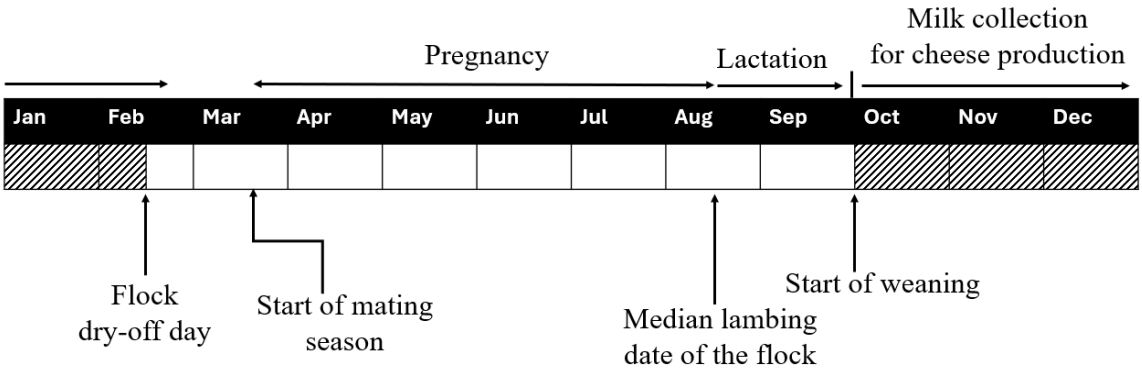


Figure 2.1. Overview of key management events, in chronological sequence, for a commercial dairy sheep flock in Masterton, New Zealand.

2.3 Dairy sheep genetics in New Zealand

The New Zealand dairy sheep flock initiated with the importation of eleven pregnant East Friesian ewes and four East Friesian rams from Sweden in 1992 (New Zealand Sheep Breeders Association 2024). Kingsmeade Farm developed the first registered New Zealand dairy sheep breed named Dairymeade. This breed originated from the crossing of East Friesian with Coopworth and Border Leicester, with subsequent breeding with East Friesians only. Animal selection has been based on temperament, health, and lactation length, and the self-replacement of ewes and dams within the flock has been in place over the last 12 years.

Maui Milk Ltd, formed in 2014, developed the Southern Cross™ breed which originated with the East Friesian, incorporated Coopworth and Awassi genetics, with more recent imports of French Lacaune semen. Their breeding programme's goal is an efficient conversion of pasture into profit, resembling the New Zealand dairy cow breeding goal. Spring Sheep Milk Co. registered the Zealandia™ breed in 2017. This breed originated from East Friesian, Lacaune, and Manech. Their breeding programme includes milk production, milk components, somatic cell count, udder conformation, and other traits. There are also breeders of pure East-Friesian and Awassi sheep in the country (New Zealand Sheep Breeders Association 2024).

In New Zealand, dairy sheep typically produce 100–150 liters per ewe per year over a 150–200-day lactation period, with a milking lifespan of 5–6 years (parities) (Ministry of Primary Industries and Massey University. 2020). In general, New Zealand dairy sheep were considered low producing compared to dairy sheep in Europe, which is a consequence of the slow rate of genetic progress for New Zealand farming systems and inadequate feed management (Lees and Lees 2018). However, the development of genetic improvement programs that are operated privately by larger companies could be changing this scenario. Nevertheless, a nationwide genetic improvement program could significantly enhance the genetics of New Zealand's dairy sheep industry, increasing production efficiency, resilience, and sustainability, with broad benefits for farms and society.

France, Italy, and Spain have established national genetic improvement programs for their local breeds of dairy sheep. These programs have led to notable improvements in milk yield, fat, and protein content over the years. The French Lacaune breed, for example, had

a genetic gain for MY (5.75 L/year) and increased contents of fat (+0.21 g/L per year) and protein (+0.14 g/L per year) from 1987 to 2001 (Astruc et al. 2002).

Highly productive breeds such as the East Friesian and Lacaune are known as "improver" breeds and have been exported to other countries for the improvement of milk yield of locally adapted breeds (Carta et al. 2009). A large difference in production performance between the different breeds of dairy sheep can be observed in Table 2.1, but differences in farming and feeding systems also influence these data. East Friesian breed, for example, leads in milk yield and lactation length whilst Lacaune leads in fat yield.

Table 2.1. Productive performance per lactation of popular breeds of dairy sheep in the world.

Breed	Lactation length	Milk yield (L)	Fat yield (kg)	Protein yield (kg)	Lactose yield (kg)	Country	Reference
Awassi	98	81	4.89	4.6	4.17	Syria	Haile et al. (2018)
East-Friesian	183	260	13.2	13.7		USA	McKusick et al. (2001)
Lacaune	152	242	21.1	16.7		France	Barrilet (2007)
Lacaune	160	181				Spain	Robles Jimenez et al. (2020)
Latxa	120	127	6.6	6.3		Spain	Legarra and Ugarte (2001)
Manchega		148	9.9	8.4		Spain	Ramon et al. (2010)
NZ dairy sheep	126	234	16.5	13		NZ	Scholtens et al. (2018)

2.4 Composition of sheep milk

Relative to cow milk, sheep milk contains higher solids, particularly fat and protein, which can be nearly double the levels in cow milk. This higher solid content offers advantages, including increased yields of processed products like cheese and enhanced nutritional benefits for humans (Chia et al. 2017). Sheep milk is also richer in essential amino acids,

unsaturated fatty acids, vitamins, and minerals (Mohapatra et al. 2019). Basic composition details of different milk species are presented in Table 2.2.

Protein, especially casein, is an important milk component as it is actively involved in milk coagulation and contributes to higher curd firmness and cheese-making ability. Fat is indirectly involved as it enhances cheese yield by being trapped in the para-casein matrix, increasing total solids recovery (Wedholm et al. 2006; Horne and Lucey 2017), these components will be discussed further.

Table 2.2. The composition of sheep milk, compared with the milks of goat and cow (Park et al. 2007; Chia et al. 2017; Mohapatra et al. 2019).

Trait	Sheep milk (%)	Goat milk (%)	Cow milk (%)
Total solids	12.1 - 20.7	11.5 - 13.5	11.5 - 14.5
Fat	6.1 - 12.6	3.4 - 4.5	3.4 - 5.5
Protein	2.6 - 6.6	2.8 - 3.7	3.2 - 4.0
Lactose	3.9 - 5.6	3.9 - 4.8	4.6 - 4.9
Water	80.7 - 82.0	83.2 - 87.0	87.2 - 87.8

2.4.1 Protein

Milk true protein is composed mainly of casein in a micellar form (in the colloidal phase of milk), and secondarily (17–22%) of whey proteins, which are present in the aqueous phase of milk (Selvaggi et al. 2014; Balthazar et al. 2017).

Casein

The casein proteins are secreted into vesicles in the mammary epithelial secretory cells and are exported through exocytosis. After exocytosis, the casein proteins assemble into spherical colloidal structures called casein micelles, a result of hydrophobic interactions, hydrogen bonds, electrostatic interactions, and colloidal calcium phosphate (CCP) nanoclusters (Honvo-Houéto et al. 2016). Casein association in the micelle is a balance between attractive and repulsive forces and several models have been proposed in the literature to describe in detail the structure of casein micelles (Huppertz 2016).

The casein micelle is a combination of calcium insensitive caseins (κ-casein) and calcium-sensitive caseins, which include α_{s1}-casein (α_{s1}-CN), α_{s2}-casein (α_{s2}-CN) and β-casein (β-CN). The calcium-sensitive caseins contain phosphoserine residues, and they are located in the interior of the micelles in association with CCP. In contrast, the κ-caseins (κ-CN) are glycosylated proteins located on the surface of the micelle. They are highly flexible and hydrophilic, forming a 'hairy layer' on the micelle surface, stabilising it against aggregation. κ-casein influences micelle size, with larger micelles occurring at lower κ-casein concentrations (Dalgleish 2011).

The proportion of each casein protein in sheep milk varies between species (Table 2.3) and across studies on sheep milk (Table 2.4; Bramanti et al. 2003; Moatsou et al. 2004; Pelmus et al. 2012; Kawęcka and Radkowska 2023). Differences might be largely attributed to the distinct sheep breeds, but the feed is also suggested to influence milk protein composition (Kawęcka and Radkowska 2023). The main fractions of sheep versus cow milk casein are 52 and 37% for α_{s1}-CN, 32 and 35% for β-CN, and 11 and 12% for κ-CN, respectively (Wendorff and Haenlein 2017). The composition of casein fraction can not only affect the technological properties of milk, but it also has implications in human health through the availability of bioactive peptides (Pereira 2014).

Casein micelles have unique structural and physicochemical properties, such as binding with ions and small molecules to form macromolecules, exceptional stabilizing characteristics, self-assembling, emulsifying, and water-binding abilities. The lack of higher protein structures (secondary and tertiary structures) is associated with the susceptibility of caseins to proteolysis, leading to curd formation. It also confers caseins with high heat stability, in contrast to the whey proteins (Fox et al. 2015). The casein micelles in sheep milk are richer in calcium compared to the micelles in cow milk, and no $CaCl_2$ additive is required for cheese-making (Balthazar et al. 2017).

The behaviour of casein micelles is pH dependent. They tighten at lower pH and swell at higher pH values. Decreasing the pH towards the isoelectric point (4.6 - 4.8) causes aggregation of casein micelles with the release of calcium, emphasizing the importance of cation accessibility for micelle formation. Conversely, an increase in pH above the isoelectric point promotes electrostatic repulsions between casein molecules, loosening the micellar structure and resulting in an increase in size (Ye and Harte 2013). The stability of casein micelles depends not only on pH range but also on the partition of milk salts, and

temperature applied, and these properties have implications in food processing (Holt et al. 2013). Overall, both the casein composition and the casein micellar structure of milk can affect the technological properties of milk.

Whey

The major whey proteins are α-lactalbumin and β-lactoglobulin, accounting (together) for 92% of whey proteins in sheep milk (Hejtmánková et al. 2012). Immunoglobulins, serum albumin, protease peptones, and lactoferrin represent a smaller part of the whey proteins. Whey proteins remain soluble in milk serum after rennet or acid precipitation of caseins (Park et al. 2007), and they can be added to beverages to increase their nutritional value. Whey proteins are more susceptible to denaturation due to their high levels of secondary and tertiary structures (Fox et al. 2015).

β-lactoglobulin (β-LG) is the main constituent whey protein in ruminant milk. β-LG accounts for 46.7-64.5% of total whey protein in sheep milk (Hejtmánková et al. 2012; Selvaggi et al. 2014). β-lactoglobulin can improve rennet coagulation and impair heat stability of milk due to its high cysteine content which, upon heat denaturation, enables it to form a covalent complex with κ-casein (Fox et al. 1998). α-lactalbumin (α-LA) is the second most abundant whey protein in milk and is involved in the biosynthesis of lactose (Fox et al. 2015).

Table 2.3. Comparison of the concentrations, in % of total protein, of the main proteins of sheep, goat, and cow milks, from New Zealand farms (Li et al. 2022; van der Zeijden et al. 2023).

Protein fraction	Sheep	Goat	Cow
Caseins			
κ-casein	11.2 ± 1.0	16.6 ± 0.7	13.1 ± 0.4
α_{s1}-casein	34.1 ± 1.1	8.9 ± 0.4	23.3 ± 0.7
α_{s2}-casein	11.9 ± 1.0	10.9 ± 0.4	4.7 ± 0.3
β-casein	29.0 ± 0.5	44.2 ± 0.8	38.2 ± 1.0
Whey proteins			
β-lactoglobulin	4.7 ± 0.5	8.3 ± 0.4	17.6 ± 1.8
α-lactalbumin	9.1 ± 0.5	11.1 ± 0.8	3.2 ± 0.1

Table 2.4. Comparison of protein concentrations, across different studies on sheep milk composition (Moatsou et al. 2004; Pelmus et al. 2012; Li et al. 2022; Kawęcka and Radkowska 2023).

[1] Protein fraction	Pelmus et al. (2012)[2] (% TP)	Kawęcka and Radkowska (2023)[3] (% TP)	Li et al. (2022)[4] (% TP)	Moatsou et al. (2004)[5] (% Casein)	Pan et al. (2023)[4] (% Casein)
Caseins	74.15				
κ-CN	17.45	4.91–7.13	11.2	9.1–10.8	7.14
α_s-CN		37.01–39.49			
α_{s1}-CN	17.38		34.1	33.9–39.9	41.91
α_{s2}-CN	14.32		11.9	12.0–16.4	16.03
β-CN	25.00	30.19–34.28	29.0	37.0–42.3	34.91
Whey	25.84				
β-LG	14.08	11.10–12.17	4.70		
α-LA	11.76	4.01–4.79	9.10		

[1]CN=casein; LG=lactoglobulin; LA=lactalbumin; TP= total protein.
[2]Romanian local sheep breed Teleorman Black Head Tsigai.
[3]Polish Mountain Sheep.
[4]New Zealand dairy sheep, bulk milk.

[5]Indigenous Greek breeds.

Polymorphisms of casein and whey proteins

Both casein and whey proteins can occur in different genetic variants, as a result of genetic polymorphisms (single nucleotide polymorphisms, SNPs). These DNA mutations lead to the presence of more than one type of allele in a locus which can result in variations in amino acid sequence. Genomic deletions and insertions (indels) can also cause protein variants (Martin et al. 2002). The genetic variants of proteins are also associated with differences in milk composition and technological properties of milk (Chianese et al. 1997; Pirisi et al. 1999; Padilla et al. 2018).

Genomic and proteomic approaches can be used to identify and characterize protein variants, respectively (Buzás et al. 2022). The protein phenotype is also affected by the level of glycosylation, for κ-CN, and phosphorylation for the calcium-sensitive caseins (Martin et al. 2002). Several sheep milk protein variants have been reported. At least 8, 7, 5, 3, and 2 variants have been reported for α_{s1}-CN (A−F,H,I), α_{s2}-CN(A−G) , β-CN (A,B,C,X,Y), β-LG (A,B,C), and α-LA (A,B), respectively (Selvaggi et al. 2014).

2.4.2 Fat

Fat is mainly present in milk in the form of fat globules, which in sheep milk are smaller than those in cow milk (Balthazar et al. 2017; Li et al. 2022), and this may have implications for digestibility. The fat globule is surrounded by the milk fat globule membrane (MFGM), a phospholipid layer (Horne and Lucey 2017). Diet can affect the fat content of milk, for example it is known that fibre-rich diets can increase fat content (Morand-Fehr et al. 2007)

Fatty acids (FAs) are the building blocks of fat, and it is well known that not only fat content but also the milk fatty acid profile is mainly affected by animal's genetics but also largely affected by diet (Addis et al. 2005; Morand-Fehr et al. 2007). Fatty acids are classified into saturated (SFA), monosaturated (MUFA), unsaturated (UFA), and polyunsaturated (PUFA). The higher prevalence of pasture feeding in New Zealand relative to other countries is suggested to contribute to lower SFA and higher PUFA in goat and sheep types of milk (Li et al. 2022).

The fatty acid profile of milk is also affected by the energy metabolism of the organism. Body fat reserves are mobilized into blood in the form of non-esterified fatty acids (NEFAs) under conditions of energy deficit (Antunović et al. 2017). However, genetics also plays some effect on the fatty acid profile of sheep milk. It has been shown that FA profile varies between breeds and genotypes (Correddu et al. 2019; Conte et al. 2022).

The fatty acid profile has implications for the sensory properties of dairy products like cheese (aroma and flavour) (Addis et al. 2005) and also some implications in texture, but mainly relevant for butter spreadability (Hurtaud et al. 2007; Horne and Lucey 2017). The FA profile of milk also has implications for human health: unsaturated FAs have previously been thought to be more desirable for human cardiovascular health, although this advice is being reconsidered considering recent evidence (Astrup et al. 2020).

2.4.3 Lactose

Lactose is the main carbohydrate in milk, which is synthesized from free glucose and galactose, and the mammary gland and the mammary gland can consume up to 85% of circulating glucose in lactating ruminants (Annison and Linzell 1964). The lactose synthesis in the mammary gland's epithelial cells is catalysed by lactose synthase, and the essential cofactor α-LA, in the Golgi compartment (Watkins and Hassid 1962).

The lactose content in ruminants' milk is lower at the beginning (colostrum) and at the end of lactation, and follows the lactation curve of milk yield, contrary to the lactation curves of fat and protein contents in the milk (Wendorff and Haenlein 2017). Lactose is the main osmotic component of milk (Pulina et al. 2005), thus its percentage should not vary as much as fat and protein percentages due to homeostasis. However, it is becoming more evident that variations in lactose percentage indicate disturbances in the mammary gland's health. Decreases in lactose percentage have been associated with increased somatic cell count (SCC) in the milk of cows (Antanaitis et al. 2021), ewes (Carta et al. 2023), and does (Podhorecká et al. 2021), and thus could be used as a biomarker in the diagnosis of mastitis. In Sarda ewes, for example, a strong negative genetic correlation between lactose and SCC (-0.94 ± 0.05) has been reported (Carta et al. 2023).

When there is epithelial damage or inflammatory processes, changes in the permeability of the blood-milk barrier occur, leading to an increased flow of soluble inorganic ions (i.e.,

K, Cl, and Na) from the blood to the milk, altering the electrical conductivity. When the osmotic pressure increases due to the elevated ion concentration (Na^+ and Cl^-), this effect is counterbalanced by a reduction in lactose concentration in milk (Stocco et al. 2019).

2.4.4 Minerals

Minerals in milk exist either in the serum phase, as free ions or dissolved salts, or bound to casein micelles, i.e. in colloidal form. Calcium phosphate, phosphorus, and magnesium are mainly associated with the casein micelles, while potassium, sodium, and chloride ions are primarily present in the serum (Gaucheron 2011). However, milk salt equilibria are dynamic systems. Factors such as pH, temperature, and protein concentration can affect the salt balance between soluble and colloidal states (Dumpler et al. 2020). Minerals such as Ca and P vary largely in proportion to the casein content of milk (Stocco et al. 2021).

Sheep milk has a higher total content of Ca, P, and Mg than cow and goat milks, but these minerals are mainly bound to the casein micelles in sheep milk (Raynal-Ljutovac et al. 2008; Li et al. 2022; Amalfitano et al. 2024). For instance, percentages of Ca, Mg, and P in the serum phase of goat milk were 29.7%, 69.2%, and 49.7%, respectively, whereas for sheep milk these values were 19.6%, 58.2%, and 38.7% (Li et al. 2022). In cow milk, soluble Ca, Mg, and P were 30%, 65%, and 54% of the total, respectively (Gaucheron 2005).

The role of mineral partitioning equilibria in the technological properties of milk is gaining increasing attention. Research has confirmed that both soluble and colloidal forms of Ca are critical factors influencing milk coagulation properties (Bauland et al. 2020). This area remains an active field of investigation in milk from ruminant species. The influence of animal genetics on milk minerals has recently gained attention in dairy cattle (Singh et al. 2024) and is an area to be explored in dairy sheep.

Table 2.5. Mineral composition of milk from goat, sheep, and cows.

	Goat[1]	Sheep[1]	Cow[2]
	Mean ± SD	Mean ± SD	Mean ± SD
Calcium (mg/100 g)	114 ± 5	200 ± 7	114 ± 14
Magnesium (mg/100 g)	14 ± 1	18 ± 2	9 ± 1
Phosphorus (mg/100 g)	100 ± 3	160 ± 3	92 ± 1
Potassium (mg/100 g)	203 ± 9	132 ± 7	146 ± 13
Sodium (mg/100 g)	37 ± 1	42 ± 7	29 ± 4
Chloride (mg/100 g)	164 ± 8	89 ± 12	-
Copper (mg/kg)	0.103 ± 0.020	0.171 ± 0.072	0.084 ± 0.058
Iodine (mg/kg)	0.251 ± 0.049	0.224 ± 0.042	0.249 ± 0.106
Selenium (mg/kg)	0.029 ± 0.004	0.036 ± 0.005	-
Zinc (mg/kg)	3.578 ± 0.286	5.822 ± 0.307	3.960 ± 0.694

[1]Values from bulk sheep milk in New Zealand, adapted from Li et al. (2022).
[2]Adapted from Toscano et al. (2023).

2.4.5 Somatic cell count

Somatic cells in milk include epithelial cells from the mammary gland and leukocytes that are transferred from the blood (Schultz 1977). Physiological increases in epithelial cells occur in milk in early (immediately post-partum) and late lactation. Leukocyte levels, however, rise during injury or mastitis infection as they combat pathogenic organisms (Sevi et al. 1999). 1999). A peculiarity related of milk secretion in sheep is that it is predominantly of apocrine type, therefore more cytoplasmic particles are present in sheep milk which translates into a higher somatic cell count (SCC) value when compared to cows, which employ merocrine type of milk secretion (Thomas and Haenlein 2017).

Although the gold standard test for mastitis infection is through bacteriological analysis, elevated SCC is routinely tested in dairy cows' milk to help identify subclinical mastitis. The occurrence of mastitis in a flock or herd can be as high as 50% of the flock causing significant milk yield reduction and economic losses (Hagnestam-Nielsen and Østergaard 2009). In addition, somatic cells may be associated with altered milk composition and poorer milk processability (Sevi et al. 1999; Pirisi et al. 2000). Greater whey protein content (lower casein:protein ratio) in milk of >1000 x 10^3 cells/mL was found and impacted its processability for cheese-making. It is also likely that plasmin, a proteolytic

enzyme produced by somatic cells such as macrophages (Raynal-Ljutovac et al. 2007b), could affect caseins. Also, levels over 400 x 10^3 cells/mL were associated with higher milk pH (+0.04), lower contents of phosphorus and potassium, and higher contents of sodium and chloride (Summer et al. 2010).

A recent study in New Zealand found the prevalence of clinical mastitis in dairy sheep to be 6% across 20 farms, with most cases occurring during early lactation (Chambers et al. 2024). Unlike dairy cow milk, there is no standardized somatic cell count (SCC) threshold for sheep milk in New Zealand. An average physiological SCC level of sheep milk, based on publications from various countries and sheep breeds, was 375 × 10^3 cells/mL, though a large variation was noted across breeds and populations (Kaskous et al. 2023). Some sources recommend a threshold of up to 500 x 10^3 cells/mL (Sutera et al. 2018), while others suggest up to 1000 × 10^3 cells/mL (Tančin et al. 2017).

2.5 Physicochemical properties of milk

Sheep milk natural pH can vary from 6.51 to 6.85 (Park et al. 2007). Milk pH is a measurement of hydrogen ion concentration in the milk and milk acidity is influenced by the level of acids and salts in milk. The acidity of milk is also related to its protein content because proteins have substantial buffering capacity. Increased titratable acidity can also indicate the accumulation of lactic acid from lactose fermentation by lactic acid bacteria. Variations of Ca^{2+}, pH, and buffering capacity from individual milk samples contribute to differences in milk stability, processability, and differences in the quality of final dairy products (Nian et al. 2012).

2.6 Processability of milk

Milk is a dynamic system characterized by the instability of its structures. Examples of this dynamism include changes in the conformation of proteins and changes in the solubility of salts with fluctuations in temperature and pH, respectively. In addition, the milk fat globule membrane and the presence of various enzymes contribute to the dynamic nature of milk. Furthermore, microorganisms can induce significant alterations in milk through the secretion of enzymes and by changing the milk pH (Fox et al. 2015).

The responses of the casein micelles during the production of dairy products such as yogurts or rennet curds or in stability situations (heat treatment) have been thoroughly reviewed (Horne 2020; Holt and Carver 2024). Despite this, the behavior of casein micelles in different experimental conditions remains an area of ongoing research (Bauland et al. 2020; Ahmadi et al. 2024; Bing et al. 2024; Deshwal et al. 2024).

The aptitude of milk to be transformed into cheese after rennet addition can be assessed through rheological studies which measure how a sample responds to applied force in terms of flow, deformation, and disintegration. Various mechanical, vibrational, ultrasonic, thermal, and optical devices are available for assessing the coagulation properties of milk (Bittante et al. 2012; Pazzola 2019). Understanding the steps of curd formation is essential, given the emphasis of the current investigation on sheep milk's suitability for cheese-making, and the historical global utilization of sheep milk in cheese manufacturing. Additionally, considering the significance of milk powder exportation by the New Zealand sheep dairy industry, a secondary emphasis is given on the ability of milk to withstand high-temperature treatment.

2.6.1 Coagulation process in cheese-making

In cheese-making, milk coagulation is mainly achieved by enzymatic reaction using rennet but can also be achieved by acidic coagulation using lactic acid bacteria cultures. For sheep milk, freezing might precede cheese-making due to the small quantity of milk produced by ewes (Wendorff and Kalit 2017), and milk is defrosted and heated (pasteurized) before cheese-making.

Rennet can be natural, i.e. a solution of milk clotting enzymes extracted from the abomasum (fourth stomach) of suckling calves or lambs. The main active enzyme in rennet is chymosin (rennin) but other important enzymes include pepsin and lipase. Alternatively, plant coagulants (Ben Amira et al. 2017) can be used or genetically modified organisms can produce recombinant chymosin (Lucey 2011; Mamo and Balasubramanian 2018). Milk rennet coagulation is described in two stages which overlap.

In the first stage, the κ-casein proteins, located on the surface of micelles, are hydrolyzed by chymosin. More specifically, κ-CN is cut into para-κ-CN, which remains in the micelle, and into glycomacropeptide, which goes into the serum. This process reduces the micelle’s

electrostatic and steric repulsion. In the second stage, aggregation of micelles occurs due to dispersion forces and calcium-induced charge shielding and salt bridging. The varying degrees of phosphorylation of the α_s- and β-caseins are reflected in the sensitivity of these molecules to calcium-induced precipitation. After sufficient destabilization and aggregation has occurred, the coagulum is formed, a three-dimensional space-filling gel (Horne and Lucey 2017).

Beyond these stages, the curd structure continues to change, and the curd is cut for expulsion of liquid (whey proteins, lactose, and water) and further firming (Lucey 2011; Horne and Lucey 2017). Most of the casein and milk fat and over half of the calcium will be retained in the final curd (cheese), therefore, cheese yield is related to the casein and fat content of the milk (Wendorff and Kalit 2017). In general, cheesemakers are more interested in the cuttability of the gel (Horne and Lucey 2017), and in cheese yield for obvious economic reasons. Good coagulating milk has a shorter rennet coagulation time and stronger gel hardness (Zhang et al. 2023).

For European protected denomination of origin cheeses, however, specific raw milk characteristics are required, such as high protein content and good renneting properties, appropriate fat content with appropriate fatty acid composition, and the presence of chemical flavors originating from local feeds. For artisan-protected denomination of origin cheeses, standardisation is not allowed, and raw milk must be used. Slight variations in texture throughout the season are allowed for protected denomination of origin cheeses (Bertoni et al. 2001). In New Zealand artisan cheese-making, pasteurisation of milk is common.

2.6.2 Factors affecting milk rennet coagulation

Several factors can affect rennet coagulation. Inherent factors include pH, casein content, casein fractions and polymorphisms, the level of casein phosphorylation, casein micelle size, ionic strength, and the distribution of calcium (soluble vs. insoluble), as well as fat globule size. Additionally, external factors such as temperature applied, the concentration of denatured whey proteins, casein hydrolysis by proteinases (which can also be inherent), enzyme concentration, and the natural lactic acid bacteria cultures in acid-coagulated cheeses play significant roles (Horne and Lucey 2017; Zhang et al. 2023). However,

assessing all inherent milk characteristics in a large-scale recording scheme for breeding purposes is likely impractical.

It is known that the optimum rennet coagulation temperature is between 30 and 35 °C. In addition, the optimal pH for the action of chymosin in milk is 6.0, and lower natural milk pH can lead to a reduction of RCT due to reduced electrostatic repulsion between micelles, solubilization of colloidal calcium phosphate and therefore increased Ca^{2+}, aggregation of caseins with less κ-casein breakdown required, and increased rennet activity (Lucey 2011).

The presence of Ca^{2+} in serum can reduce rennet coagulation time and increase the rate of curd firming due to neutralization of the negative charge on the micelle surface, weakening repulsion and accelerating coagulation (Tsioulpas et al. 2007). The rennet clotting time of milk also depends on micelle size; with small caseins having the shortest clotting time and higher gel strength (Glantz et al. 2010). As fat is entrapped in the curd, fat content might also affect coagulum firmness (Wendorff and Kalit 2017).

Overall, the high concentration of protein, fat, and calcium per casein unit in sheep milk makes it an ideal raw material for cheese production (Moatsou et al. 2004). A higher ratio of κ-CN to total casein is linked to firmer curds (Zhang et al. 2023). Sheep milk also has higher β/α_s-casein ratio, which makes coagulation proceed faster than in cow milk (Muir et al. 1993a).

2.6.3 Heat treatment of milk

Although sheep milk can be excellent for cheese-making, it is known to have lower stability under heat treatments when compared with cow milk (Pan et al. 2023). This is suggested to be due to its high concentration of solids, and other factors related to the sheep casein micelle properties (size, mineralization, and composition), easier denaturation of whey proteins (Raynal-Ljutovac et al. 2007a), and higher ionic calcium concentration (Pan et al. 2023). Milk heat-stability is highly pH dependent and has been reported as a function of milk pH. Muir et al. (1993b) reported maximal heat stability (18 minutes) of skim sheep milk at pH 6.78. Whereas (Pan et al. 2023) reported maximal skim sheep milk stability (10 minutes) at pH 6.9, with heat stability reducing at higher or lower pH values. For dairy cows, Fox and Hoynes (1976) reported maximum heat stability (20 - 30 min) at pH 6.7.

Heating milk above 100 ºC causes several simultaneous reactions including dissociation of κ-casein from micelles, shifts in the salt equilibrium, acidification, dephosphorylation of proteins and proteolysis, which collectively drive to protein coagulation (Fox et al. 1998). The extent of changes depends on temperature applied, heating time, pH, ionic strength, and protein concentration (Dumpler et al. 2020). It is known that larger proportions of κ-casein, higher calcium, lower citrate and phosphate content, and lower proportions of urea reduce milk heat stability of bovine milk (Timlin et al. 2021).

It is widely accepted that temperature increases significantly decrease milk pH. This is mainly attributed to heat-induced precipitation of tertiary calcium phosphate (Pouliot et al. 1989). Also, in prolonged heating, organic acids are formed from the heat-induced degradation of lactose, further reducing milk pH (Berg and Boekel 1994). At temperatures above 100 °C, κ-caseins dissociate from the surface of the micelle leading to protein aggregation. The level of dissociation is pH-dependent (Anema 1998), as very low heat stability was observed at low pH (Singh and Fox, 1985). During heat treatment, whey proteins suffer denaturation and can associate with the micellar κ-caseins, this association can be reversible or irreversible depending on the conditions of the heat treatment and compositional factors (Walstra et al., 2005; Wijayanti et al., 2014). Sheep milk behavior to high heat treatment could possibly be compared to that of concentrated bovine milk, whose heat stability is negatively affected by naturally occurring higher concentrations of whey proteins, especially of β-lactoglobulin (Muir and Sweetsur 1978).

2.7 Factors affecting ewes' milk yield, composition, and physicochemical properties

Milk production and raw milk composition and physicochemical properties can be influenced by several factors. These factors can be physiological, such as the ewe's stage of lactation, parity number, litter characteristics, metabolic and health status (Jaramillo et al. 2008; Kuchtík et al. 2008; Hejtmánková et al. 2012; Pazzola et al. 2014; Hunter et al. 2015; Vacca et al. 2015; Manca et al. 2016; Abecia and Palacios 2017; Sutera et al. 2018). Genetic factors, such as breed, variety, and genotype, are also known to affect the lactation curves of ewes (Peralta-Lailson et al. 2005; Padilla et al. 2018; Robles Jimenez et al. 2020; Velarde-Guillén et al. 2022). Environmental factors, such as mating management, lambing season, feeding strategies, diet, and milking practices, can further influence these lactation curves (Sevi et al. 2004; Morand-Fehr et al. 2007; Bliss 2018; Abecia et al. 2020). Some

of these factors are often included in statistical models to allow for proper adjustments and assessment of the genetic component affecting ewes' performance.

In seasonal grazing systems, fluctuating weather conditions significantly impact pasture quality and availability, thereby affecting milk production, composition, and processability. In such systems, the stage of lactation often coincides with specific times of the year, making it challenging to separate these factors (McCoard et al. 2023). Periods of inadequate feeding may compromise milk yield due to direct and indirect effects of nutrient availability. Glucose and amino acid availability are vital for milk synthesis, as they serve as the primary building blocks for mammary epithelial cells. Glucose can stimulate lactose synthesis and promote cell proliferation in mammary epithelial cells (Lin et al. 2016). To support optimal milk production, diets must provide sufficient energy and nitrogen for rumen microbes, as well as essential amino acids in rumen-protected form (Gross 2023).

Additionally, an improved nutritional state can influence the IGF-I response, which partly explains the positive effects on milk yield and coagulation properties (Pulina and Nudda 2004). Milk secretion is regulated by endocrine factors (hormones). Milk yield gradually declines after peak production as lactation progresses, until the mammary gland undergoes involution. This process involves the apoptosis of mammary epithelial cells and is associated with decreasing levels of prolactin, growth hormone (GH), and IGF-I (Svennersten-Sjaunja and Olsson 2005). Changes in the expression of glucose transporters also occur during different lactational stages (Gross 2022).

The physiology of lactation, combined with feeding, and metabolic status are reflected in changes in milk composition and processability throughout the milking season. As milk yield reduces, milk becomes more concentrated. Also, the ratio of casein to protein can change, a higher content of ovine acid whey proteins was noticed at the beginning and the end of lactation (Hejtmánková et al. 2012). Milk pH has been reported to increase in late lactation (Sevi et al. 2004) or reduce in mid-lactation (Kuchtík et al. 2008), and udder health to decrease as lactation progresses (Manca et al. 2016). Rennet coagulation time has been shown to increase (Sevi et al. 2004; Jaramillo et al. 2008) or decrease (Manca et al. 2016) with the progress of lactation or reduce in mid-lactation (Kuchtík et al. 2008; Pazzola et al. 2014). Likewise, curd firmness has also been shown to increase with lactation (Vacca et al. 2015) or decrease (Sevi et al. 2004).

Although natural variations in milk composition can be minimized through bulking of milk (Nian et al. 2012), and protein content can be standardized within the dairy industry (Horne and Lucey 2017), it remains crucial to ensure that a high proportion of milk is inherently well-suited for processing. This not only reduces milk losses but also improves quality. For artisan cheese production, in particular, milk must be optimal for processing from the source, usually with no major modifications other than acidity level, temperature, and enzyme concentration. Natural fluctuations in milk quality should not exceed the cheesemaker's skill to adapt to them (Bertoni et al. 2001).

2.8 Genetics of milk production, composition, and processability in dairy sheep

Animals must possess the genetic potential to achieve high performance within a farming system for the operation to be profitable. While management strategies, like improved feeding, can enhance an animal's production level, these are limited by its inherent genetic capacity. Additionally, matching genetics to the production system will lead to more efficient production by reducing husbandry and feeding costs (Flint and Woolliams 2008).

The decision of replacement dams and sires of the next generation should be carefully considered. In animal breeding, an animal's estimated breeding worth (BW) is used for ranking animals (Lopez-Villalobos and Garrick 2005) and thus assists in selection. The BW is calculated using an index that assigns weight to economically important traits. Heritability plays a key role in calculating estimated breeding values of traits, as it reflects the degree to which a trait can be passed from one generation to the next (Flint and Woolliams 2008). Moreover, understanding the correlations between traits helps predict how selection for one trait may influence the genetic progress of another trait. This knowledge ensures more informed and effective selection decisions (Lopez-Villalobos 2012).

Milk processability can be genetically improved in two primary ways, represented in Figure 2.2. The first approach would target specific genes proven to significantly affect milk traits. The second is a quantitative genetic approach, which involves the estimation of an animal's breeding value for a processability trait based on pedigree (ancestry) data, which can be combined with genomic information such as SNP data for improved accuracies (Barillet 2007; Bittante et al. 2012).

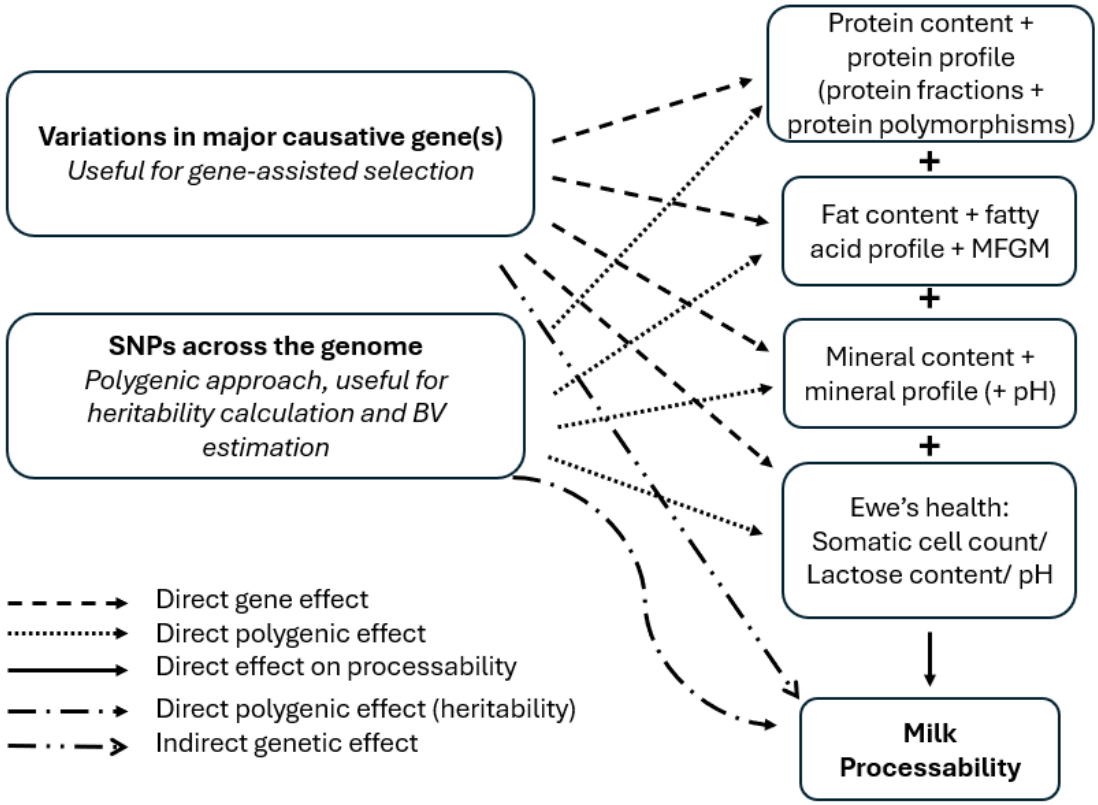


Figure 2.2. Schematic representation of how specific genes or multiple genes (polygenic effects) could be affecting milk processability (adapted from Bittante et al. 2012).

2.8.1 Heritability estimates

Some studies conducted overseas have reported heritability estimates (h^2) of milk production (Hamann et al. 2004; Haile et al. 2018; Sánchez-Mayor et al. 2019; Mucha et al. 2022), milk composition (Bittante et al. 2017; Pazzola et al. 2018; Carta et al. 2023), and cheese-making traits, including milk coagulation properties, individual laboratory cheese yield, and occurrence of non-coagulating milk in dairy sheep (Othmane et al. 2002; Bittante et al. 2017; Puledda et al. 2017; Sánchez-Mayor et al. 2019; Pelayo et al. 2021; Correddu et al. 2022; Gaspa et al. 2022a).

Table 2.6. Heritability estimates reported in the literature for milk production and composition traits[1] in dairy sheep.

Test-day yields									
Reference	Breed	MY	FY	PY	LY	FP	PP	LP	SCS/SCC[1]
Bittante et al. (2017)	Sarda	0.04	-	-	-	0.18	0.18	-	-
De la Fuente et al. (2011)	Churra	0.06	-	-	-	0.04	0.10	-	0.09
Hamann et al. (2004)	East-Friesian	0.15	0.15	0.15	-	0.09	0.20	-	0.16
Legarra and Ugarte (2005)	Latxa	0.21	-	-	-	-	-	-	0.13
Othmane et al. (2002)	Churra	0.15	-	-	-	0.06	0.23	-	0.11
Pelayo et al. (2021)	Churra	0.17	-	-	-	0.42	0.30	-	0.22
Puledda et al. (2017)	Sarda	0.08	-	-	-	0.10	0.13	-	0.03
Sánchez-Mayor et al. (2019)	Spanish Assaf	0.34	0.02	0.25	0.32	0.04	0.33	0.18	0.21
Total lactation yields									
Reference	Breed	MY	FY	PY	LY	FP	PP	LP	SCS/SCC[1]
Barillet (2007)	Lacaune	0.32	0.26	0.28	-	0.41	0.51	-	0.15
Carta et al. (2023)	Sarda	-	-	-	-	-	-	0.03	-
Gutiérrez et al. (2007)	Spanish Assaf	0.11	-	-	-	-	-	-	-
Haile et al. (2018)	Awassi	0.29	0.30	0.20	0.26	-	-	-	-
Makovický et al. (2018)	Slovak dairy sheep	0.27	-	-	-	-	-	-	0.07
Legarra and Ugarte (2001)	Latxa	0·20	0·16	0·18		0·14	0·38	-	-
Mucha et al. (2022)	Multiple breeds	0.24	0.21	0.22	-	0.28	0.33	-	0.13

[1]MY= milk yield; FP= fat percentage; PP= protein percentage; FY= fat yield; PY= protein yield; LY= lactose yield. [1]Reported either as somatic cell score (SCS) or somatic cell count (SCC), the formulas for SCS differ across studies.

Table 2.7. Heritability estimates reported in the literature for cheese yield, milk coagulation properties, and milk pH.

		ILCY	MCP			pH
Reference	Breed		RCT	K20	A30	
Bittante et al. (2017)	Sarda	-	0.16	0.10	0.08	0.15
Puledda et al. (2017)	Sarda	0.16	0.23	0.15	0.14	0.16
Othmane et al. (2002)	Churra	0.08	-	-	-	-
Sánchez-Mayor et al. (2019)	Spanish Assaf	0.30	0.22	0.29	0.30	0.46
Pelayo et al. (2021)	Churra	0.15	0.22	0.26	0.16	0.34

ILCY= individual laboratory cheese yield (%); MCP= milk coagulation properties; RCT= rennet coagulation time (min); K20= time to reach curd firmness of 20 mm (min); A30= curd firmness at 30 minutes (mm).

However, a large variation in heritability estimates is noted in the results obtained from different dairy sheep breeds or populations, presented in Tables 2.6 and 2.7. Heritability values for milk yield ranged from as low as 0.04 for Sarda and 0.34 for Spanish Assaf sheep (Bittante et al. 2017; Sánchez-Mayor et al. 2019). This variation could be attributed to reduced genetic variation in populations that have undergone intense artificial selection for specific dairy traits, as seen in Sarda sheep, which leads to lower heritability estimates (Lush 1949).

These findings highlight the importance of conducting genetic studies within populations or with populations that are genetically alike. Also, heritability estimates for milk composition traits can range from low to high in different breeds of sheep (from 0.04 to 0.51) (Barrilet et al. 2005; De la Fuente et al. 2011). Only recently heritability estimates for lactose percentage are being reported for dairy sheep (Carta et al. 2023).

Milk coagulation traits exhibit low to moderate heritability values in dairy sheep (0.08 to 0.30), as seen in Table 2.7, which could be mainly due to their susceptibility to environmental influences such as analytical methods. However, moderate heritability values still provide opportunities for genetic improvement, allowing these traits to be effectively exploited in breeding programs. This was also true for dairy cows, as the heritability estimates averaged from across the literature were 0.26 for RCT, 0.36 for K20, and 0.27 for A30. The high average repeatability estimates for MCP in dairy cows

(0.50−0.60) also meant that sampling could be done on only a few occasions throughout lactation (Bittante et al. 2012).

Few studies have investigated the genetic correlations between MCP and milk composition in dairy sheep, and most of them were done in Sarda, Table 2.8. High protein percentage was genetically correlated to longer RCT (Bittante et al. 2017; Puledda et al. 2017), and stronger A30 (Pelayo et al. 2021). Higher fat percentage was related to stronger A30 (Bittante et al. 2017; Puledda et al. 2017), but the opposite was also reported (Pelayo et al. 2021). Lactose percentage was genetically correlated with short RCT and K20 (Pazzola et al. 2018). Stronger correlations were found between milk pH and MCP. High milk pH was genetically correlated with longer RCT and K20, and softer A30 (Puledda et al. 2017; Sánchez-Mayor et al. 2019; Pelayo et al. 2021).

Furthermore, inconsistent results are reported on genetic correlations between SCS and MCP (Puledda et al. 2017; Pazzola et al. 2018), and between ILCY and MCP. Curd firmness was negatively correlated with individual cheese yield in Sarda (Puledda et al. 2017) but positively correlated in Spanish Assaf sheep (Sánchez-Mayor et al. 2019). Individual cheese yield was correlated with longer RCT and K20 (Puledda et al. 2017; Sánchez-Mayor et al. 2019), phenotypic correlations followed the same trend.

These differences may be from different breeds, milk composition, farming systems, sample sizes, and statistical methods. Although the literature on genetic correlations is more extensive for dairy cattle, the findings cannot be directly compared to that of sheep milk due to the much higher concentration of milk solids and other unique physicochemical properties of sheep milk.

Table 2.8. Genetic (below diagonal) and phenotypic (above diagonal) correlations between milk production, composition, SCS, pH, coagulation traits, and cheese yield, reported in the literature.

[1] Trait	MY	FP	PP	LP	SCS
MY		-0.43[a]	-0.41[a]	0.39[f]	-0.16[b], -0.14[a]
FP	-0.63[a], -0.35[g]		0.62[a]	-0.32	0.04[a], 0.11[b]
PP	-0.68[a], -0.65[g]	0.81[a],0.51[g]		-0.30	0.13[a], 0.21[b]
LP	0.79[f]	-0.35[b]	-0.36[b]		-0.49[b]
SCS	-0.36[a], 0.13[b]	0.04[a], 0.06[b]	0.13[a],0.18[b]	-0.88[b]	
pH	-0.22[b],0.21[g]	-0.17[g], -0.00 [b]	-0.26[g], 0.41[b]	-0.27[b]	0.44[b], 0.58[g]
RCT	-0.03[d], 0.03[c], 0.06[g]	-0.11[g], -0.02[c], 0.13[d]	0.18[g], 0.41[c], 0.76[d]	-0.47[b]	-0.14[c], 0.62[b], 0.84[g]
K20	-0.44[d],0.04[c], 0.42[g]	-0.34[c], -0.30[g] -0.12[d]	-0.42[c], -0.24[g], -0.05[d]	-0.57[b]	-0.72[c], 0.59[b], 0.83[g]
A30	-0.51[g], -0.41[d], 0.27[c]	-0.32[g], 0.32[c], 0.33[d]	-0.01[d], 0.09[c], 0.26[g]	0.03[b]	-0.47[b], -0.92[g], 0.11[c]
ILCY	-0.88[c], -0.36[a], -0.33[g]	0.45[c], 0.60[a], 0.74[g]	0.68[g], 0.75[c], 0.76[a]	-	0.10[g], 0.58[c]

[1] Trait	pH	RCT	K20	A30	ILCY
MY	-0.18[b]	-0.09[c], -0.06[d]	-0.06[d], 0.07[c]	-0.04[c], -0.01[d]	-0.20[a], -0.09[c]
FP	0.03[b]	0.09[c], 0.19[d]	-0.06[d], 0.03[c]	-0.12[c], 0.16[d]	0.37[a], 0.46[c]
PP	0.06[b]	0.30[c], 0.37[d]	-0.04[c], -0.20[d]	0.03[c], 0.42[d]	0.31[a], 0.37[c]
LP	-0.40[b]	-0.51[b]	-0.29[b]	0.09[b]	-
SCS	0.35[b]	0.45[c], 0.48[b]	0.27[b], 0.35[c]	-0.30[c], -0.04[b]	0.35[c]
pH		0.68[e], 0.70[c]	0.55[c], 0.61[e]	-0.56[e], -0.42[c]	0.11[e], 0.18[c]
RCT	0.68[c], 0.75[g], 0.81[e]		0.65[e], 0.69[d], 0.79[c]	-0.94[e], -0.60[c], -0.12[d]	0.12[e], 0.41[c]
K20	0.44[c], 0.72[e], 0.87[g]	0.75[d], 0.84[c], 0.87[e], 0.88[g]		-0.76[c], -0.69[e], -0.49[d]	0.01[e], 0.32[c]
A30	-0.83[c], -0.68[e], -0.68[g]	-0.93[e], -0.80[c], -0.77[g], -0.55[d]	-0.93[g], -0.91[c], -0.90[e] -0.72[d]		-0.34[c], 0.01[e]
ILCY	0.06[e],0.09[g], 0.58[c]	0.13[g], 0.35[e], 0.55[c]	-0.03[g], 0.04[e], 0.64[c]	-0.67[c], 0.04[g], 0.17[e]	

[a]Othmane et al. (2002), Churra; [b]Pazzola et al. (2018), Sarda; [c]Puledda et al. (2017), Sarda; [d]Bittante et al. (2017), Sarda; [e]Sánchez-Mayor et al. (2019), Spanish Assaf. [f] Carta et al. (2023), Sarda; [g]Pelayo et al. (2021), Churra.

[1]MY= test-day milk yield; FP= fat percentage; PP= protein percentage; SCS= somatic cell score, [$Log_2(SCC \times 10^{-5}) + 3$], and calculated as Log_{10} SCC in Pelayo et al. (2021); RCT= rennet coagulation time (min); K20= time to reach curd firmness of 20 mm (min); A30= curd firmness at 30 minutes (mm); ILCY= individual laboratory cheese yield (%).

2.8.2 Genes associated with sheep milk production, composition, and processability

Genomic information, usually in the form of SNP data, can be used, combined with ancestry data, either for a more accurate estimation of the breeding values of individuals or for genome-wide association studies (GWAS), which provide an understanding of the genetic architecture of traits and try to find genes that could be causative (Table 2.9). Proven genes could then be implemented in gene-assisted selection if they have a large enough effect on the trait and can be applied across populations and breeds (Johnsson 2023).

Identification of true causative genes for gene-assisted selection, however, can be difficult due to the polygenic nature of most production traits, linkage disequilibrium, and epistasis. Usually, to confirm GWAS findings, a group of genes with the highest number of signals (significant SNP associations for the trait of interest) are selected for full sequencing using PCR, and a simpler statistical test for the association is performed (Ramos et al. 2009; Corral et al. 2010; Padilla et al. 2018; Dettori et al. 2020). Before the popularization of GWAS, studies focused on finding variations in specific genes such as the casein genes or β-LG, mainly, without assessing the functional meaning of those variations (Chianese et al. 1996; Chessa et al. 2010; Othman et al. 2013).

The use of genomic breeding values estimation and gene-assisted selection for some traits have already been commonly used in nucleus meat sheep flocks in New Zealand. In sheep, genomic tools are important due to the limited use of AI, and DNA parentage testing helps keep track of pedigree as well as allows breeding value predictions for animals that have not been phenotyped, based on information from a training population that has phenotypic records on traits that are difficult to measure (McEwan 2009; Johnsson 2023).

Reports of GWAS on dairy sheep milk traits are relatively recent compared to that of dairy cows, and the knowledge in this area, particularly concerning milk processability traits, is still building up. Understanding the genome architecture is an additional means of confirming trends found in genetic correlation estimates.

Several genes have been associated with milk production, composition, and processability in sheep, as can be seen in Table 2.9. The differing results reported across studies so far could be attributed to various factors, including differences in trait definitions, within-breed linkage disequilibrium, variations in allele frequencies across breeds, pleiotropic effects, biased SNP selection on the genotyping arrays, population sub-structuring, or

sequencing errors (Qanbari 2020; Gaspa et al. 2022b). However, some agreement across GWAS is notable, especially regarding the importance of CSN1S1, CSN1S2, CSN2, CSN3 (Chr 6), GH, GHR, GHRHR (Chr 11, 16, 4), LALBA (Chr 3), SCD (Chr 22), SLC27A6 (Chr 5), SLC35A2 (Chr X), TTC7B (Chr 7) for dairy sheep milk traits.

2.9 Summary and implications

There is still no selection index for the wider New Zealand dairy sheep industry and there have been no specific public research programs targeted at dairy sheep genetics, leaving this critical aspect mainly to the private sector. The national dairy sheep flock requires animals that are well-suited to New Zealand's farming conditions, especially since the use of specialized dairy breeds originated from overseas intensive systems has historically proven not to be fit for New Zealand's pasture-based farming, environment, and climate conditions.

Obtaining sheep milk production, composition, and processability data is expensive, often making it unjustifiable for smaller businesses to record ewes' performance. However, it is possible that larger companies can utilize gene-assisted selection or employ genomic breeding value predictions with single nucleotide polymorphism information.

A significant gap in research remains. No studies have investigated the genetic and genomic foundations of milk production, composition, and processability within New Zealand dairy sheep. Despite the high use of sheep milk for processing, technological traits are not recorded or considered in animal breeding programs. The large variation of genetic and genomic results across studies overseas highlights the need for investigation within New Zealand. Such research could provide valuable insights and help the industry develop more effective breeding strategies.

Table 2.9. Candidate genes most significantly associated with milk traits in sheep, through genome-wide association.

[1]Trait(s)	Candidate gene(s) abbreviation	Candidate gene's full name	Chr	Reference, breed, country
Milk composition	ACACA	acetyl-CoA carboxylase alpha	11	Marina et al. (2020): multiple breeds worldwide
Non-coagulation	ALKBH6	alkB homolog 6	14	Gaspa et al. (2022b): Sarda, Italy
Dairy production	ABCG2	ATP binding cassette subfamily G member 2	6	Gutierrez-Gil et al. (2014): multiple European breeds
MCP, ILCY, ILDCY, MY, FP, PP, LP, SCS, pH	CD44	CD44 molecule	15	Marina et al. (2021): Assaf and Churra, Spain
MY, FY, PY	CDH13	Cadherin 13	14	Li et al. (2020): East-Friesian x Lacaune cross, USA
SCC , non-coagulation	CHKA	Choline kinase alpha	21	Gaspa et al. (2022b): Sarda, Italy
Non-coagulation	CMPK1	Cytidine/uridine monophosphate kinase 1	1	Gaspa et al. (2022b): Sarda, Italy
MY, FY, PY	CNTN4	Contactin 4	19	Li et al. (2020): East-Friesian x Lacaune cross, USA
MY, PP	CSN1S1, CSN1S2, CSN2, CSN3	Casein alpha s1, Casein alpha s2, Casein beta, Casein kappa	6	Usai et al. (2019): Lacaune, Sarda, and its crosses, Europe, Marina et al. (2020): multiple breeds worldwide

SCC	EGFLAM	EGF like, fibronectin type III and laminin G domains	16	Marina et al. (2021): Assaf and Churra, Spain
SCS	FAM49A	CYFIP related Rac1 interactor A	3	Sutera et al. (2021): Valle del Belice sheep, Italy
Dairy production	FKBP4	FKBP prolyl isomerase 4	3	Gutierrez-Gil et al. (2014): multiple European breeds
SCS	FPGT	ucose-1-phosphate guanylyltransferase	1	Sutera et al. (2021): Valle del Belice sheep, Italy
MY, FY, PY	GALNT14	polypeptide N-acetylgalactosaminyltransferase 14	3	Li et al. (2020): East-Friesian x Lacaune cross, USA
MY	GH, GHR, GHRHR	Growth hormone , Growth hormone receptor, Growth hormone releasing hormone receptor	11, 16, 4	Gutierrez-Gil et al. (2014): multiple European breeds, Usai et al. (2019): Lacaune, Sarda, and its crosses, Europe
SCS	IGF2R	Insulin like growth factor 2 receptor	8	Sutera et al. (2021): Valle del Belice sheep, Italy
MCP, ILCY, ILDCY, MY, FP, PP, LP, SCS, pH	ITPR1	Inositol 1,4,5-trisphosphate receptor type 1	19	Marina et al. (2021): Assaf and Churra, Spain
MY, FY, PY	ITPR2	Inositol 1,4,5-trisphosphate receptor type 2	3	Li et al. (2020): East-Friesian x Lacaune cross, USA
PP, FP	LALBA	Lactalbumin alpha	3	Usai et al. (2019): Lacaune, Sarda, and its crosses, Europe, Marina et al. (2020): multiple breeds worldwide, Garcia-Gamez et al. (2012): Churra, Spain

MY, FY, PY	LRP1B	LDL receptor related protein 1	2	Li et al. (2020): East-Friesian x Lacaune cross, USA
Non-coagulation	LRRC41	Leucine rich repeat containing 41	1	Gaspa et al. (2022b): Sarda, Italy
SCS	LRRIQ3	Leucine rich repeats and IQ motif containing 3	1	Sutera et al. (2021): Valle del Belice sheep, Italy
MY, FP, PP	MECOM	MDS1 and EVI1 complex locus	1	Marina et al. (2021): Assaf and Churra, Spain
SCS	NEGR1	Neuronal growth regulator 1	1	Sutera et al. (2021): Valle del Belice sheep, Italy
MY, PP	PAEP	Progestagen associated endometrial protein	3	Marina et al. (2020): multiple breeds worldwide
MCP, ILCY, ILDCY, MY, FP, PP, LP, SCS, pH	PCSK2	Proprotein convertase subtilisin/kexin type 2	13	Marina et al. (2021): Assaf and Churra, Spain
MY	PDZRN4	PDZ domain containing ring finger 4	3	Costilla et al. (2023): New Zealand dairy sheep, NZ
Milk composition	PLIN2	Perilipin 2	2	Marina et al. (2020): multiple breeds worldwide
Dairy production	POFUT1	Protein O-fucosyltransferase 1	13	Gutierrez-Gil et al. (2014): multiple European breeds
Non-coagulation	PPIL3	Peptidylprolyl isomerase like 3	2	Gaspa et al. (2022b): Sarda, Italy
Litter mean weight at weaning	PRLR	Prolactin receptor		Esmaeili-Fard et al. (2021): Baluchi sheep, Iran

Milk composition	SCD	Stearoyl-CoA desaturase	22	Marina et al. (2020): multiple breeds worldwide, Gutierrez-Gil et al. (2014): multiple European breeds
SCS	SERP1	Stress associated endoplasmic reticulum protein 1	1	Sutera et al. (2021): Valle del Belice sheep, Italy
MCP, ILCY, ILDCY, MY, FP, PP, LP, SCS, pH	SLC20A2	Solute carrier family 20 member 2	26	Marina et al. (2021): Assaf and Churra, Spain
MY, FY, PY , Milk composition	SLC27A6	Solute carrier family 27 member 6	5	Marina et al. (2020): multiple breeds worldwide, Li et al. (2020): East-Friesian x Lacaune cross, USA
Lactose, Non-coagulation	SLC35A2	Solute carrier family 35 member A2	X	Gaspa et al. (2022b): Sarda, Italy, Usai et al. (2019): Lacaune, Sarda, and its crosses, Europe
MY, FY, PY	SLC39A12	Solute carrier family 39 member 12	13	Li et al. (2020): East-Friesian x Lacaune cross, USA
SCS	SOD2	Superoxide dismutase 2	8	Sutera et al. (2021): Valle del Belice sheep, Italy
Non-coagulation	SPCS3	Signal peptidase complex subunit 3	26	Gaspa et al. (2022b): Sarda, Italy
Milk production	SPP1	Secreted phosphoprotein 1	6	Gutierrez-Gil et al. (2014): multiple European breeds
FP, PP	SUCNR1	Succinate receptor 1	1	Sutera et al. (2019): Valle del Belice sheep, Italy
Non-coagulation	SYNE4	Spectrin repeat containing nuclear envelope family member 4	14	Gaspa et al. (2022b): Sarda, Italy
Non-coagulation	TCIRG1	T cell immune regulator 1, ATPase H+ transporting V0 subunit a3	21	Gaspa et al. (2022b): Sarda, Italy
MY, FP, PP	TFAP2C	Transcription factor AP-2 gamma	13	Usai et al. (2019): Lacaune, Sarda, and its crosses, Europe

MY, FY, PY, FP, PP	TTC7B	Tetratricopeptide repeat domain 7B	7	Sutera et al. (2019): Valle del Belice sheep, Italy, Li et al. (2020): East-Friesian x Lacaune cross, USA
Milk composition	XDH	Xanthine dehydrogenase	3	Marina et al. (2020): multiple breeds worldwide
Non-coagulation	ZCCHC17	Zinc finger CCHC-type containing 17	2	Gaspa et al. (2022b): Sarda, Italy
MY, FP, PP	ZFPM1	Zinc finger protein, FOG family member 1	14	Marina et al. (2021): Assaf and Churra, Spain
MY, FP, PP	ZNF250	Zinc finger protein 250	9	Marina et al. (2021): Assaf and Churra, Spain

[1]MY= milk yield; FY= fat yield; PY=protein yield; FP= fat percentage; PP= protein percentage; LP= lactose percentage; SCS= somatic cell score; SCC= somatic cell count; MCP= milk coagulation properties; ILCY= individual laboratory cheese yield; ILDCY= individual laboratory dry cheese yield.

References

Abecia J, Palacios C. 2017. Ewes giving birth to female lambs produce more milk than ewes giving birth to male lambs. Italian Journal of Animal Science. 17: 736−739.

Abecia JA, Garcia A, Castillo L, Palacios C. 2020. The effects of weather on milk production in dairy sheep vary by month of lambing and lactation phase. Journal of Animal Behaviour and Biometeorology. 5: 56−63.

Addis M, Cabiddu A, Pinna G, Decandia M, Piredda G, Pirisi A, Molle G. 2005. Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid cis-9, trans-11. Journal of Dairy Science. 88: 3443−3454.

Ahmadi E, Huppertz T, Vasiljevic T. 2024. Heat stability of skim milk containing various levels of micellar calcium phosphate. International Dairy Journal. 153: 105900.

Amalfitano N, Patel N, Haddi M-L, Benabid H, Pazzola M, Vacca GM, Tagliapietra F, Schiavon S, Bittante G. 2024. Detailed mineral profile of milk, whey, and cheese from cows, buffaloes, goats, ewes and dromedary camels, and efficiency of recovery of minerals in their cheese. Journal of Dairy Science. Pre-proof vailable online on 3 July 2024.

Anema SG. 1998. Effect of milk concentration on heat-induced, pH-dependent dissociation of casein from micelles in reconstituted skim milk at temperatures between 20 and 120 C. Journal of Agricultural and Food Chemistry. 46: 2299−2305.

Annison E, Linzell J. 1964. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their over-all metabolism and to milk formation. The Journal of Physiology. 175: 372.

Antanaitis R, Juozaitienė V, Jonike V, Baumgartner W, Paulauskas A. 2021. Milk lactose as a biomarker of subclinical mastitis in dairy cows. Animals. 11: 1736.

Antunović Z, Novoselec J, Šperanda M, Steiner Z, Ćavar S, Pavlović N, Lendić KV, Mioč B, Paćinovski N, Klir Ž. 2017. Monitoring of blood metabolic profile and milk quality of ewes during lactation in organic farming. Mljekarstvo/Dairy. 67.

Astruc J, Barillet F, Barbat A, Clément V, Boichard D 2002. Genetic evaluation of dairy sheep in France. Proceedings of the Seventh World Congress on Genetics Applied to Livestock Production, Montpellier, France, CDROM Communication.

Astrup A, Magkos F, Bier DM, Brenna JT, de Oliveira Otto MC, Hill JO, ... Krauss RM. 2020. Saturated fats and health: a reassessment and proposal for food-based recommendations: JACC state-of-the-art review. Journal of the American College of Cardiology. 76: 844−857.

Balthazar C, Pimentel T, Ferrão L, Almada C, Santillo A, Albenzio M, Mollakhalili N, Mortazavian A, Nascimento J, Silva M. 2017. Sheep milk: physicochemical characteristics and relevance for functional food development. Comprehensive Reviews in Food Science and Food Safety. 16: 247−262.

Barillet F. 2007. Genetic improvement for dairy production in sheep and goats. Small Ruminant Research. 70: 60−75.

Barrilet F, Arranz J, Carta A. 2005. Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep. Genetics Selection Evolution. 37: 109−121.

Bauland J, Famelart M-H, Bouhallab S, Jeantet R, Roustel S, Faiveley M, Croguennec T. 2020. Addition of calcium and magnesium chlorides as simple means of varying bound and precipitated minerals in casein micelle: Effect on enzymatic coagulation. Journal of Dairy Science. 103: 9923−9935.

Ben Amira A, Besbes S, Attia H, Blecker C. 2017. Milk-clotting properties of plant rennets and their enzymatic, rheological, and sensory role in cheese making: A review. International Journal of Food Properties. 20: S76−S93.

Berg H, Boekel Mv. 1994. Degradation of lactose during heating of milk. 1. Reaction pathways. Netherlands Milk and Dairy Journal. 48: 157-175.

Bertoni G, Calamari L, Maianti MG. 2001. Producing specific milks for speciality cheeses. Proceedings of the Nutrition Society. 60: 231−246.

Bhutto RA, Fan Y, Kang L, Wang M, Iqbal S, Yi J. 2024. Bovine α-Lactalbumin: Source, extraction, techno-functional properties, and applications as a (nano-) delivery system for nutraceuticals. Trends in Food Science and Technology. 104381.

Bing H, Hong X, Tao X, Liu D, Zhang J, Yang T, Liu TC, Liu X, Zhou P. 2024. Structure and digestibility of bovine casein complexes formed by enriching κ-and β-caseins in micellar casein concentrate together with minerals adjustment. International Dairy Journal. 105939.

Bittante G, Cipolat-Gotet C, Pazzola M, Dettori M, Vacca G, Cecchinato A. 2017. Genetic analysis of coagulation properties, curd firming modeling, milk yield, composition, and acidity in Sarda dairy sheep. Journal of Dairy Science. 100: 385−394.

Bittante G, Penasa M, Cecchinato A. 2012. Invited review: Genetics and modeling of milk coagulation properties. Journal of Dairy Science. 95: 6843−6870.

Bliss AE 2018. An investigation into the effects of different housing and feeding systems on behaviour and milk production of dairy ewes in mid and late stages of lactation : a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Animal Science at Massey University, Manawatu, New Zealand.

Bramanti E, Sortino C, Onor M, Beni F, Raspi G. 2003. Separation and determination of denatured α_{s1}-, α_{s2}-, β-and κ-caseins by hydrophobic interaction chromatography in cows', ewes' and goats' milk, milk mixtures and cheeses. Journal of Chromatography A. 994: 59−74.

Buzás H, Székelyhidi R, Szafner G, Szabó K, Süle J, Bukovics S, Kovács AJ. 2022. Developed rapid and simple RP-HPLC method for simultaneous separation and quantification of bovine milk protein fractions and their genetic variants. Analytical Biochemistry. 658: 114939.

Carta A, Casu S, Salaris S. 2009. Invited review: Current state of genetic improvement in dairy sheep. Journal of Dairy Science. 92: 5814−5833.

Carta S, Cesarani A, Correddu F, Macciotta N. 2023. Understanding the phenotypic and genetic background of the lactose content in Sarda dairy sheep. Journal of Dairy Science. 106: 3312−3320.

Chambers G, Laven R, Grinberg A, Ridler A, Velathanthiri N. 2024. An observational study of farmer-reported clinical mastitis in New Zealand dairy ewes. New Zealand Veterinary Journal. 72: 1−13.

Chessa S, Rignanese D, Berbenni M, Ceriotti G, Martini M, Pagnacco G, Caroli A. 2010. New genetic polymorphisms within ovine β-and α_{s2}-caseins. Small Ruminant Research. 88: 84−88.

Chia J, Burrow K, Carne A, McConnell M, Samuelsson L, Day L, Young W, Bekhit AE-DA 2017. Minerals in sheep milk. Nutrients in Dairy and their Implications on Health and Disease, Elsevier. Pp. 345−362.

Chianese L, Garro G, Mauriello R, Laezza P, Ferranti P, Addeo F. 1996. Occurrence of five α_{s1}-casein variants in ovine milk. Journal of Dairy Research. 63: 49−59.

Chianese L, Mauriello R, Ferranti P, Tripaldi C, Taibi L, Dell'Aquila S. 1997. Relationship between alpha$_{s1}$-casein variants and clotting capability of ovine milk. In: Milk

protein polymorphism. Proceedings of the IDF Seminar, Palmerston North, New Zealand, February 1997.

Conte G, Palombo V, Serra A, Correddu F, D'Andrea M, Macciotta NPP, Mele M. 2022. Study of the fatty acid profile of milk in different sheep breeds: evaluation by Multivariate Factorial Analysis. Animals 12: 722.

Corral J, Padilla J, Izquierdo M. 2010. Associations between milk protein genetic polymorphisms and milk production traits in Merino sheep breed. Livestock Science. 129: 73−79.

Correddu F, Cellesi M, Serdino J, Manca MG, Contu M, Dimauro C, Ibba I, Macciotta NPP. 2019. Genetic parameters of milk fatty acid profile in sheep: comparison between gas chromatographic measurements and Fourier-transform IR spectroscopy predictions. Animal. 13: 469−476.

Correddu F, Gaspa G, Cesarani A, Macciotta N. 2022. Phenotypic and genetic characterization of the occurrence of noncoagulating milk in dairy sheep. Journal of Dairy Science. 105: 6773−6782.

Costilla R, Chan P, McRae K, McEwan J, Dodds K, Newman S-A, Baird H, Henry H, Brauning R, Cullen N. 2023. Genome-wide association studies for milk traits in New Zealand dairy sheep. New Zealand Journal of Animal Science and Production. 82: 64−68.

Dalgleish DG. 2011. On the structural models of bovine casein micelles-review and possible improvements. Soft Matter. 7: 2265−2272.

De la Fuente F, Gonzalo C, Sánchez J, Rodríguez R, Carriedo J, Primitivo F. 2011. Genetic parameters of the linear body conformation traits and genetic correlations with udder traits, milk yield and composition, and somatic cell count in dairy ewes. Canadian Journal of Animal Science. 91: 585-591.

Deshwal GK, Fenelon M, Gómez-Mascaraque LG, Huppertz T. 2024. Influence of citrate- and phosphate-based calcium sequestering salts on the disruption of casein micelles. Food Hydrocolloids. 153: 109970.

Dettori ML, Pazzola M, Petretto E, Vacca GM. 2020. Association analysis between SPP1, POFUT1 and PRLR gene variation and milk yield, composition and coagulation traits in Sarda sheep. Animals. 10: 1216.

Dumpler J, Huppertz T, Kulozik U. 2020. Invited review: Heat stability of milk and concentrated milk: Past, present, and future research objectives. Journal of Dairy Science. 103: 10986−11007.

Esmaeili-Fard SM, Gholizadeh M, Hafezian SH, Abdollahi-Arpanahi R. 2021. Genes and pathways affecting sheep productivity traits: genetic parameters, genome-wide association mapping, and pathway enrichment analysis. Frontiers in Genetics. 12: 710613.

FAO. 2022. Production of raw milk of sheep: top 10 producers. Available at: https://www.fao.org/faostat/en/#data/QCL/visualize (accessed on 02 October 2024).

Flint A, Woolliams J. 2008. Precision animal breeding. Philosophical Transactions of the Royal Society B: Biological Sciences. 363: 573−590.

Fonterra Co-operative Group Limited. 2024. Farmgate Milk Price. Available at: https://www.fonterra.com/nz/en/investors/farmgate-milk-price.html (accessed on 02 October 2024).

Fox P, Hoynes M. 1976. Heat stability characteristics of ovine, caprine and equine milks. Journal of Dairy Research. 43: 433−442.

Fox P, Uniacke-Lowe T, McSweeney P, O'Mahony J. 2015. Milk proteins. In: Dairy Chemistry and Biochemistry, Springer.

Fox P, McSweeney P. 1998. Heterogeneity of milk proteins. In: Dairy Chemistry and Biochemistry, Springer.

Garcia-Gamez E, Gutierrez-Gil B, Sahana G, Sanchez J-P, Bayon Y, Arranz J-J. 2012. GWA analysis for milk production traits in dairy sheep and genetic support for a QTN influencing milk protein percentage in the LALBA gene. Plos One. 7: e47782.

Gaspa G, Cesarani A, Correddu F, Congiu M, Dimauro C, Pauciullo A, Macciotta N 2022a. Heritability and genomic analysis of coagulation event in sheep milk. Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP) Technical and species orientated innovations in animal breeding, and contribution of genetics to solving societal challenges. Pp. 2928−2931.

Gaspa G, Correddu F, Cesarani A, Congiu M, Dimauro C, Pauciullo A, Macciotta NPP. 2022b. Multivariate and Genome-Wide Analysis of Mid-Infrared Spectra of Non-Coagulating Milk of Sarda Sheep Breed. Frontiers in Animal Science. 3: 889797.

Gaucheron F. 2005. The minerals of milk. Reproduction Nutrition Development 45: 473-483.

Gaucheron F. 2011. Milk and dairy products: a unique micronutrient combination. Journal of the American College of Nutrition. 30: 400S−409S.

Glantz M, Devold T, Vegarud G, Månsson HL, Stålhammar H, Paulsson M. 2010. Importance of casein micelle size and milk composition for milk gelation. Journal of Dairy Science. 93: 1444−1451.

Griffiths L. 2015. Business plan for the NZ sheep dairy industry. Available at: https://ruralleaders.co.nz/business-plan-for-the-nz-sheep-dairy-industry-lucy-griffiths-cruickshank/ (accessed on 02 October 2024).

Gross JJ. 2022. Limiting factors for milk production in dairy cows: perspectives from physiology and nutrition. Journal of Animal Science. 100: skac044.

Gross JJ. 2023. Dairy cow physiology and production limits. Animal Frontiers: The Review Magazine of Animal Agriculture. 13: 44−50.

Gutierrez-Gil B, Arranz JJ, Pong-Wong R, Garcia-Gamez E, Kijas J, Wiener P. 2014. Application of selection mapping to identify genomic regions associated with dairy production in sheep. Plos One. 9: e94623.

Hagnestam-Nielsen C, Østergaard S. 2009. Economic impact of clinical mastitis in a dairy herd assessed by stochastic simulation using different methods to model yield losses. Animal. 3: 315−328.

Haile A, Hilali M, Hassen H, Lobo R, Rischkowsky B. 2018. Estimates of genetic parameters and genetic trends for growth, reproduction, milk production and milk composition traits of Awassi sheep. Animal. 13: 240−247.

Hamann H, Horstick A, Wessels A, Distl O. 2004. Estimation of genetic parameters for test day milk production, somatic cell score and litter size at birth in East Friesian ewes. Livestock Production Science. 87: 153−160.

Hejtmánková A, Pivec V, Trnková E, Dragounová H. 2012. Differences in the composition of total and whey proteins in goat and ewe milk and their changes throughout the lactation period. Czech Journal of Animal Science. 57: 323−31.

Holt C, Carver J, Ecroyd H, Thorn D. 2013. Invited review: Caseins and the casein micelle: Their biological functions, structures, and behavior in foods. Journal of Dairy Science. 96: 6127−6146.

Holt C, Carver J. 2024. Invited review: Modeling milk stability. Journal of Dairy Science.

Honvo-Houéto E, Henry C, Chat S, Layani S, Truchet S. 2016. The endoplasmic reticulum and casein-containing vesicles contribute to milk fat globule membrane. Molecular Biology of the Cel.l 27: 2946−2964.

Horne DS, Lucey JA. 2017. Rennet-induced coagulation of milk. Cheese: 115−143.
Horne DS. 2020. Casein micelle structure and stability. Milk Proteins, Elsevier. Pp. 213−250.
Hunter T, Suster D, DiGiacomo K, Dunshea F, Cummins L, Egan A, Leury B. 2015. Milk production and body composition of single-bearing East Friesian× Romney and Border Leicester× Merino ewes. Small Ruminant Research 131: 123−129.
Huppertz T. 2016. Heat stability of milk. Advanced dairy chemistry: volume 1B: proteins: Applied Aspects: 179−196.
Hurtaud C, Delaby L, Peyraud J-L. 2007. The nature of preserved forage changes butter organoleptic properties. Le Lait 87: 505−519.
Jaramillo D, Zamora A, Guamis B, Rodríguez M, Trujillo A. 2008. Cheesemaking aptitude of two Spanish dairy ewe breeds: Changes during lactation and relationship between physico-chemical and technological properties. Small Ruminant Research 78: 48−55.
Johnsson M. 2023. Genomics in animal breeding from the perspectives of matrices and molecules. Hereditas 160: 20.
Kaskous S, Farschtschi S, Pfaffl MW. 2023. Physiological Aspects of Milk Somatic Cell Count in Small Ruminants—A Review. Dairy 4: 26−42.
Kawęcka A, Radkowska I. 2023. Comparison of the quality of mountain sheep milk obtained from animals kept on a natural and organic mountain pasture. Annals of Animal Science 23: 275−283.
Kilgour R, Waterhouse T, Dwyer C, Ivanov I. 2008. Farming systems for sheep production and their effect on welfare. The welfare of sheep, Springer. Pp. 213−265.
Kuchtík J, Sustova K, Urban T, Zapletal D. 2008. Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. Czech Journal of Animal Science 53: 55.
Lees N, Lees I. 2018. Competitive advantage through responsible innovation in the New Zealand sheep dairy industry. International food and agribusiness management review 21: 505−524.
Li H, Wu XL, Tait Jr R, Bauck S, Thomas D, Murphy T, Rosa G. 2020. Genome-wide association study of milk production traits in a crossbred dairy sheep population using three statistical models. Animal Genetics 51: 624−628.
Li S, Delger M, Dave A, Singh H, Ye A. 2022. Seasonal variations in the composition and physicochemical characteristics of sheep and goat milks. Foods 11: 1737.
Lin Y, Sun X, Hou X, Qu B, Gao X, Li Q. 2016. Effects of glucose on lactose synthesis in mammary epithelial cells from dairy cow. BMC Veterinary Research 12: 1−11.
Lopez-Villalobos N, Garrick D. 2005. Methodology for the design and enhancement of genetic improvement programs illustrated in the context of the New Zealand dairy industry. Agrociencia 9: 553−568.
Lopez-Villalobos N. 2012. Analysing the genetic basis of milk production traits. CABI Reviews: 1−18.
Lush JL. 1949. Heritability of quantitative characters in farm animals. Proceedings of the 8th International Congress of Genetics, 1948. p. 356–375.
Makovický P, Margetín M, Nagy M, Makovický P. 2018. Genetic parameters for milkability and somatic cell score in Slovak dairy sheep. The Indian Journal of Animal Sciences. 88:860−862
Mamo A, Balasubramanian N. 2018. Calf rennet production and its performance optimization. Journal of Applied and Natural Science. 10: 247−252.

Manca M, Serdino J, Gaspa G, Urgeghe P, Ibba I, Contu M, Fresi P, Macciotta N. 2016. Derivation of multivariate indices of milk composition, coagulation properties, and individual cheese yield in dairy sheep. Journal of Dairy Science. 99: 4547−4557.

Marina H, Gutiérrez-Gil B, Esteban-Blanco C, Suárez-Vega A, Pelayo R, Arranz JJ. 2020. Analysis of whole genome resequencing datasets from a worldwide sample of sheep breeds to identify potential causal mutations influencing milk composition traits. Animals. 10: 1542.

Marina H, Pelayo R, Suárez-Vega A, Gutiérrez-Gil B, Esteban-Blanco C, Arranz J. 2021. Genome-wide association studies (GWAS) and post-GWAS analyses for technological traits in Assaf and Churra dairy breeds. Journal of Dairy Science. 104: 11850−11866.

Martin P, Szymanowska M, Zwierzchowski L, Leroux C. 2002. The impact of genetic polymorphisms on the protein composition of ruminant milks. Reproduction Nutrition Development. 42: 433−459.

McCoard S, Stevens D, Selbie D, Day L, Young W, Bekhit AE-D, Samuelsson L. 2023. Supporting the growth of the dairy sheep industry in New Zealand–industry update and review of a programme linking industry and science. New Zealand Journal of Agricultural Research. Published online: 14 Dec 2023.

McEwan J. 2009. Current and future impact of DNA technologies on the New Zealand sheep industry. Proceedings of the New Zealand Society of Animal Production. Christchurch, New Zealand Society of Animal Production. Pp. 165−168.

Ministry of Primary Industries and Massey University. 2020. New Zealand Sheep Dairy Survey. Wellington and Palmerston North: MPI-Massey University.

Moatsou G, Samolada M, Katsabeki A, Anifantakis E. 2004. Casein fraction of ovine milk from indigenous Greek breeds. Le Lait. 84: 285−296.

Mohapatra A, Shinde AK, Singh R. 2019. Sheep milk: A pertinent functional food. Small Ruminant Research. 181: 6−11.

Morand-Fehr P, Fedele V, Decandia M, Le Frileux Y. 2007. Influence of farming and feeding systems on composition and quality of goat and sheep milk. Small Ruminant Research. 68: 20−34.

Morris ST. 2017. Overview of sheep production systems. Advances in Sheep Welfare, Elsevier. Pp. 19−35.

Mucha S, Tortereau F, Doeschl-Wilson A, Rupp R, Conington J. 2022. Animal Board Invited Review: Meta-analysis of genetic parameters for resilience and efficiency traits in goats and sheep. Animal. 16: 100456.

Muir D, Horne D, Law A, Sweetsur A. 1993b. Ovine milk. 2. Seasonal changes in indices of stability. Milchwissenschaft. 48: 442−445 .

Muir D, Horne D, Law Ar, Sweetsur Am. 1993a. Ovine milk. II: Seasonal changes in indices of stability. Milchwissenschaft. 48: 442−445.

Muir D, Sweetsur A. 1978. The effect of concentration on the heat stability of skim-milk. Journal of Dairy Research. 45: 37−45.

Murray S. 2024. High pet ownership in China contributes to turnaround in sheep milk fortunes. Radio New Zealand. Available at: https://www.rnz.co.nz/news/country/521157/high-pet-ownership-in-china-contributes-to-turnaround-in-sheep-milk-fortunes (accessed on 02 October 2024).

New Zealand Sheep Breeders Association. 2024. Sheep breeds. Available at: https://nzsheep.co.nz/sheep-breeds/ (accessed on 02 October 2024).

Nian Y, Chen BY, Aikman P, Grandison A, Lewis M. 2012. Naturally occurring variations in milk pH and ionic calcium and their effects on some properties and processing characteristics of milk. International Journal of Dairy Technology. 65: 490−497.

Othman OE, El-Fiky SA, Hassan NA, Mahfouz ER, Balabel EA. 2013. Genetic variations of β-and K-casein genes in Egyptian sheep breeds. Journal of Applied Biosciences. 64: 4858–4866.

Othmane M, De la Fuente L, Carriedo J, San Primitivo F. 2002. Heritability and genetic correlations of test day milk yield and composition, individual laboratory cheese yield, and somatic cell count for dairy ewes. Journal of Dairy Science. 85: 2692−2698.

Padilla P, Izquierdo M, Martínez-Trancón M, Parejo JC, Rabasco A, Salazar J, Padilla JÁ. 2018. Polymorphisms of α-lactoalbumin, β-lactoglobulin and prolactin genes are highly associated with milk composition traits in Spanish Merino sheep. Livestock Science. 217: 26−29.

Pan Z, Ye A, Fraser K, Dave A, Singh H. 2023. Heat stability of sheep's skim milk: Aggregation and interaction of proteins. International Dairy Journal. 144: 105689.

Park Y, Juárez M, Ramos M, Haenlein G. 2007. Physico-chemical characteristics of goat and sheep milk. Small Ruminant Research. 68: 88−113.

Pazzola M, Cipolat-Gotet C, Bittante G, Cecchinato A, Dettori ML, Vacca GM. 2018. Phenotypic and genetic relationships between indicators of the mammary gland health status and milk composition, coagulation, and curd firming in dairy sheep. Journal of Dairy Science. 101: 3164−3175.

Pazzola M, Dettori ML, Cipolat-Gotet C, Cecchinato A, Bittante G, Vacca GM. 2014. Phenotypic factors affecting coagulation properties of milk from Sarda ewes. Journal of Dairy Science. 97: 7247−7257.

Pazzola M. 2019. Coagulation Traits of Sheep and Goat Milk. Animals (Basel) 9.

Pelayo R, Gutiérrez-Gil B, Garzón A, Esteban-Blanco C, Marina H, Arranz J. 2021. Estimation of genetic parameters for cheese-making traits in Spanish Churra sheep. Journal of Dairy Science. 104: 3250−3260.

Pelmus RS, C PG, Lazar C, Marin DE, Gras M, Radu M, Ghita E. 2012. Preliminary study on milk composition and milk protein polymorphism in the Romanian local sheep breed Teleorman Black Head Tsigai. Romanian Biotechnological Letters. 17: 7583.

Peralta-Lailson M, Trejo-González AÁ, Pedraza-Villagómez P, Berruecos-Villalobos JM, Vasquez CG. 2005. Factors affecting milk yield and lactation curve fitting in the creole sheep of Chiapas-Mexico. Small Ruminant Research. 58: 265−273.

Pereira PC. 2014. Milk nutritional composition and its role in human health. Nutrition. 30: 619−627.

Peterson S, Prichard C. 2015. The sheep dairy industry in New Zealand: A review. New Zealand Society of Animal Production. Pp. 119−126.

Peterson S. 2017. Producing quality milk from sheep. Achieving sustainable production of sheep, Burleigh Dodds Science Publishing. Pp. 95−134.

Pirisi A, Piredda G, Corona M, Pes M, Pintus S, Ledda A. 2000. Influence of somatic cell count on ewe's milk composition, cheese yield and cheese quality. Proceedings of Sixth Great Lakes Dairy Sheep Symposium, Guelph, Canada. Pp. 47−59.

Pirisi A, Piredda G, Papoff CM, Di Salvo R, Pintus S, Garro G, Ferranti P, Chianese L. 1999. Effects of sheep α_{s1}-casein CC, CD and DD genotypes on milk composition and cheesemaking properties. Journal of Dairy Research. 66: 409−419.

Podhorecká K, Borková M, Šulc M, Seydlová R, Dragounová H, Švejcarová M, Peroutková J, Elich O. 2021. Somatic cell count in goat milk: An indirect quality indicator. Foods. 10: 1046.

Pouliot Y, Boulet M, Paquin P. 1989. Observations on the heat-induced salt balance changes in milk I. Effect of heating time between 4 and 90 C. Journal of Dairy Research. 56: 185−192.

Puledda A, Gaspa G, Manca MG, Serdino J, Urgeghe PP, Dimauro C, Negrini R, Macciotta NPP. 2017. Estimates of heritability and genetic correlations for milk coagulation properties and individual laboratory cheese yield in Sarda ewes. Animal. 11: 920−928.

Pulina G, Milán M, Lavín M, Theodoridis A, Morin E, Capote J, Thomas D, Francesconi A, Caja G. 2018. Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. Journal of Dairy Science. 101: 6715−6729.

Pulina G, Nudda A. 2004. Milk production. Dairy sheep nutrition, CABI Publishing Wallingford UK. Pp. 1−12.

Qanbari S. 2020. On the extent of linkage disequilibrium in the genome of farm animals. Frontiers in Genetics. 10: 1304.

Radio New Zealand. 2024a. Seeking a slice of the US cheese market: 'It's far bigger than people realise'. Available at: https://www.rnz.co.nz/news/country/520567/seeking-a-slice-of-the-us-cheese-market-it-s-far-bigger-than-people-realise (accessed on 02 October 2024).

Radio New Zealand. 2024b. A New Zealand sheep milk company scores a big win in China. Available at: https://www.rnz.co.nz/news/country/522774/a-new-zealand-sheep-milk-company-scores-a-big-win-in-china (accessed on 02 October 2024).

Radio New Zealand. 2024c. Shock as Maui Milk tells suppliers to stop milking. Available at: https://www.rnz.co.nz/news/country/510587/shock-as-maui-milk-tells-suppliers-to-stop-milking (accessed on 02 October 2024).

Ramos A, Matos C, Russo-Almeida P, Bettencourt C, Matos J, Martins A, Pinheiro C, Rangel-Figueiredo T. 2009. Candidate genes for milk production traits in Portuguese dairy sheep. Small Ruminant Research. 82: 117−121.

Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I, Chilliard Y. 2008. Composition of goat and sheep milk products: An update. Small Ruminant Research. 79: 57−72.

Raynal-Ljutovac K, Park Y, Gaucheron F, Bouhallab S. 2007a. Heat stability and enzymatic modifications of goat and sheep milk. Small Ruminant Research. 68: 207−220.

Raynal-Ljutovac K, Pirisi A, De Cremoux R, Gonzalo C. 2007b. Somatic cells of goat and sheep milk: Analytical, sanitary, productive and technological aspects. Small Ruminant Research. 68: 126−144.

Robles Jimenez LE, Angeles Hernandez JC, Palacios C, Abecia JA, Naranjo A, Osorio Avalos J, Gonzalez-Ronquillo M. 2020. Milk production of Lacaune sheep with different degrees of crossing with Manchega sheep in a commercial flock in Spain. Animals. 10: 520.

Sánchez-Mayor M, Pong-Wong R, Gutiérrez-Gil B, Garzón A, De la Fuente L, Arranz J. 2019. Phenotypic and genetic parameter estimates of cheese-making traits and their relationships with milk production, composition and functional traits in Spanish Assaf sheep. Livestock Science. 228: 76−83.

Scale Up. 2022. Spring sheep milk co. sustainable food and fibre futures fund programme.

Schultz LH. 1977. Somatic cells in milk-physiological aspects and relationship to amount and composition of milk. Journal of Food Protection. 40: 125−131.

Selvaggi M, Laudadio V, Dario C, Tufarelli V. 2014. Investigating the genetic polymorphism of sheep milk proteins: a useful tool for dairy production. Journal of the Science of Food and Agriculture. 94: 3090−3099.

Sevi A, Albenzio M, Marino R, Santillo A, Muscio A. 2004. Effects of lambing season and stage of lactation on ewe milk quality. Small Ruminant Research. 51: 251−259.

Sevi A, Albenzio M, Taibi L, Dantone D, Massa S, Annicchiarico G. 1999. Changes of somatic cell count through lactation and their effects on nutritional, renneting and bacteriological characteristics of ewe's milk. Advances in Food Sciences. 21: 122−127.

Singh A, Kumar A, Thakur MS, Khare V, Jain A, Tiwari SP. 2024. Genetic analysis of milk minerals in dairy cattle: a review. Journal of Applied Genetics. 65: 375-381.

Sneddon N, Lopez-Villalobos N, Hickson R, Shalloo L. 2013. Review of milk payment systems to identify the component value of lactose. Proceedings of the New Zealand Society of Animal Production. Pp. 33−36.

Stocco G, Pazzola M, Dettori ML, Cipolat-Gotet C, Summer A, Vacca GM. 2019. Variation in caprine milk composition and coagulation as affected by udder health indicators. International Dairy Journal. 98: 9−16.

Stocco G, Summer A, Cipolat-Gotet C, Malacarne M, Cecchinato A, Amalfitano N, Bittante G. 2021. The mineral profile affects the coagulation pattern and cheese-making efficiency of bovine milk. Journal of Dairy Science. 104: 8439−8453.

Summer A, Franceschi P, Malacarne M, Formaggioni P, Tosi F, Tedeschi G, Mariani P. 2010. Influence of somatic cell count on mineral content and salt equilibria of milk. Italian Journal of Animal Science. 8: 435−437.

Sutera A, Riggio V, Mastrangelo S, Di Gerlando R, Sardina M, Pong-Wong R, Tolone M, Portolano B. 2019. Genome-wide association studies for milk production traits in Valle del Belice sheep using repeated measures. Animal Genetics. 50: 311−314.

Sutera AM, Moscarelli A, Mastrangelo S, Sardina MT, Di Gerlando R, Portolano B, Tolone M. 2021. Genome-wide association study identifies new candidate markers for somatic cells score in a local dairy sheep. Frontiers in Genetics. 12: 409.

Sutera AM, Portolano B, Di Gerlando R, Sardina MT, Mastrangelo S, Tolone M. 2018. Determination of milk production losses and variations of fat and protein percentages according to different levels of somatic cell count in Valle del Belice dairy sheep. Small Ruminant Research. 162: 39−42.

Svennersten-Sjaunja K, Olsson K. 2005. Endocrinology of milk production. Domestic Animal Endocrinology. 29: 241−258.

Tančin V, Baranovič Š, Uhrinčať M, Mačuhová L, Vršková M, Oravcová M. 2017. Somatic cell counts in raw ewes' milk in dairy practice: frequency of distribution and possible effect on milk yield and composition. Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka. 67: 253−260.

Thomas DL, Haenlein GF. 2017. Sheep Milk. Handbook of Milk of Non-Bovine Mammals.

Timlin M, Tobin JT, Brodkorb A, Murphy EG, Dillon P, Hennessy D, O'Donovan M, Pierce KM, O'Callaghan TF. 2021. The impact of seasonality in pasture-based production systems on milk composition and functionality. Foods. 10: 607.

Toscano A, Giannuzzi D, Pegolo S, Vanzin A, Bisutti V, Gallo L, Trevisi E, Cecchinato A, Schiavon S. 2023. Associations between the detailed milk mineral profile, milk composition, and metabolic status in Holstein cows. Journal of Dairy Science. 106: 6577−6591.

Tsioulpas A, Lewis MJ, Grandison AS. 2007. Effect of minerals on casein micelle stability of cows' milk. Journal of Dairy Research. 74: 167−173.

Usai MG, Casu S, Sechi T, Salaris SL, Miari S, Sechi S, Carta P, Carta A. 2019. Mapping genomic regions affecting milk traits in Sarda sheep by using the OvineSNP50

Beadchip and principal components to perform combined linkage and linkage disequilibrium analysis. Genetics Selection Evolution. 51: 1−19.

Vacca G, Cipolat-Gotet C, Paschino P, Casu S, Usai M, Bittante G, Pazzola M. 2019. Variation of milk technological properties in sheep milk: Relationships among composition, coagulation and cheese-making traits. International Dairy Journal. 97: 5−14.

Vacca GM, Pazzola M, Dettori ML, Pira E, Malchiodi F, Cipolat-Gotet C, Cecchinato A, Bittante G. 2015. Modeling of coagulation, curd firming, and syneresis of milk from Sarda ewes. Journal of Dairy Science. 98: 2245−2259.

van der Zeijden M, Ellis A, Lopez-Villalobos N, Li S, Roy NC, McNabb W. 2023. The protein composition of bovine milk from once-a-day and twice-a-day milking production systems in New Zealand. Dairy. 4: 689−703.

Velarde-Guillén J, López-Villalobos N, Sainz-Ramírez A, González-Sánchez M, Arriaga-Jordán CM, Albarrán-Portillo B. 2022. Factors affecting the lactation curve parameters of crossbred dairy ewes in the highlands of Mexico. Tropical Animal Health and Production. 54: 373.

Watkins WM, Hassid W. 1962. The synthesis of lactose by particulate enzyme preparations from guinea pig and bovine mammary glands. Journal of Biological Chemistry. 237: 1432−1440.

Wedholm A, Larsen LB, Lindmark-Månsson H, Karlsson A, Andrén A. 2006. Effect of protein composition on the cheese-making properties of milk from individual dairy cows. Journal of Dairy Science. 89: 3296−3305.

Wendorff W, Haenlein GF. 2017. Sheep milk–composition and nutrition. Handbook of Milk of Non-bovine Mammals: 210−221.

Wendorff W, Kalit S. 2017. Processing of sheep milk. Handbook of Milk of Non-Bovine Mammals: 222−260.

Ye R, Harte F. 2013. Casein maps: effect of ethanol, pH, temperature, and CaCl2 on the particle size of reconstituted casein micelles. Journal of Dairy Science. 96: 799−805.

Zhang W, Zheng S, Gao P, Ren Q, Zhang Y, Chen B, Hettinga K, Pang X, Lv J, Zhang S. 2023. Identification of the coagulation properties of Chinese Holstein bovine milk: Effects of milk compositions, milk protein polymorphism, and phosphorylation levels on milk coagulation ability. Food Hydrocolloids. 145: 109112

Chapter 3

Modelling lactation curves for dairy sheep in a New Zealand flock

This chapter has been published elsewhere. It has been reformatted and presented here with permission:

Marshall AC, Lopez-Villalobos N, Loveday SM, Ellis A, McNabb W. 2023. Modelling lactation curves for dairy sheep in a New Zealand flock. Animals. 13*:* 349. https://doi.org/10.3390/ani13030349

Abstract

Lactation curves were modelled for dairy sheep in a New Zealand flock, providing information on the lactation yields of milk, fat, protein, and lactose, corrected for 130 days of milking. From 169 ewes, a total of 622 test-day records were obtained during the milk production season of 2021–2022 (from October to January). The flock produced an average of 86.1 kg of milk, 5.1 kg of fat, 4.5 kg of protein, and 4.1 kg of lactose per ewe, and moderate to large coefficients of variation were observed (27–31%) for these traits. The lactation persistency of milk, fat, protein, and lactose yields ranged from 52.3 to 72.7%. Analyses of variance for total yield and persistency were performed with an animal model that included the fixed effects of age (parity number), litter size, coat colour, and milking frequency (days in twice-a-day milking) and random residuals. Age and milking frequency were the only factors that significantly affected the yields of milk, fat, protein, and lactose. Age significantly affected the lactation persistency of milk and lactose yields, whereas litter size affected the persistency of protein, and milking frequency affected the persistency of fat. This study on this single flock provides valuable experience for a larger-scale animal breeding programme in New Zealand.

Keywords

Dairy sheep; Animal model; Lactation curve; Milk production; Lactation persistency.

3.1 Introduction

The dairy industry in New Zealand is characterised by being pasture-based, with no housing and low supplementation (Lees and Lees 2018). The dairy sheep industry is expanding, and it is expected to reach NZ$750 million in annual export receipts by 2035 (O'Connor 2022). Milking sheep is potentially gentler to the environment than traditional dairy cow farming (Downie-Melrose 2014). In addition, dairy sheep milk contains more solids than cow milk and provides several health benefits to consumers due to its composition (Balthazar et al. 2017).

A small number of studies have described the milk production of dairy sheep in some New Zealand flocks (Peterson et al. 2005; McMillan et al. 2014; Bliss et al. 2018; Scholtens et al. 2018), reporting production levels that are lower than dairy sheep in well-advanced production systems in Europe. This has been suggested to be due to the small pool of

genetics that were initially imported from Europe, where animals were originally selected for intensive farming systems (Lees and Lees 2018).

Test-day records can be used to predict the total amounts of milk, fat, protein, and lactose produced by each animal throughout lactation. Test-day records are taken at fixed dates, but individual animals will be on different days in milk because they started producing at different dates. For example, a single test-day might be on day 20 of the lactation for an animal that gave birth 20 days ago, and simultaneously, it is day 45 for an animal that gave birth 45 days ago. These animals are not in the same stage of lactation and are not comparable, given that the stage of lactation significantly affects milk yield and composition (Kuchtík et al. 2008). One way to overcome this problem and estimate total yields for individual animals is through the modelling of the lactation curve using random regression with Legendre polynomials (Schaeffer 2004; Macciotta et al. 2005; Silvestre et al. 2006). This technique models the covariance between repeated records taken on the same animal over time and allows the prediction of variances and covariances for timepoints along the trajectory, even though few observations are made, but using information from all other measurements (Van der Werf et al. 1997; Kominakis et al. 2001). This approach is considered ideally suited for the analysis of longitudinal data in animal breeding (Schaeffer 2004; Kirkpatrick et al. 1990).

Lactation curves can be used to calculate lactation persistency, which indicates the ability of animals to maintain a reasonably constant milk yield after peak production. Several methods for the calculation of lactation persistency have been proposed, but there is still no standard method (Danell 1982; Sölkner and Fuchs 1987; Swalve and Gengler 1999).

The total yield obtained for each animal can be used for the analysis of variance. The explanatory variables or factors that are known to affect milk performance are included as fixed (e.g., breed and parity number) or random (e.g., herd and animal) effects. Several factors that may affect milk production and the shape of the lactation curve include genetics (such as the breed and individual genotype), physiological factors (such as age or parity, live weight, health, and offspring characteristics such as litter size), and farm management (such as milking frequency and animal nutrition) (Bencini and Pulina 1997).

The objective of this study was to model the lactation curves of dairy sheep in a New Zealand commercial flock and identify the factors affecting the shape of the lactation curve and the total yields and persistency of milk, fat, protein, and lactose yields.

3.2 Materials and methods

Data were collected from 169 Dairymeade ewes at Kingsmeade Farm, Masterton, New Zealand. Animal ethics approval was obtained for this study (Massey University Animal Ethics Committee-Protocol 21/45). The breed was established in 1996, initially using Coopworth and Border Leicester dams and semen from European East Friesian sires. The resulting progenies were subsequently mated with only East Friesian sires (Lopez-Villalobos et al. 2017). The farm has been using the self-replacement of ewes and rams over the past 12 years. In Dairymeade ewes, only two colour variants are visually distinguished, white and black.

The farm has 11 hectares of land, is located on flat land, and operates an extensive seasonal pasture-based system; the ewes have limited access to supplementary feed during milking. Rams are left to mate with mixed-age ewes (over two years old) in mid-March, with ewe hoggets in mid-April and the lambing season starting in mid-August. The farm has an exclusive suckling period, so milking for artisan cheese production starts after the lambs are fully weaned when they reach 13.5 kg liveweight (at the discretion of the farmer). In the 2021–2022 season, the average suckling period was 57 days.

Masterton has a mild temperate climate that resembles a Mediterranean climate; warm dry settled weather predominates in summer, and frosts may occur in winter. In the 2021–2022 season, the maximum temperatures ranged from 24.7 °C to 30.2 °C, with January being the hottest month. Minimum temperatures ranged from −0.2 °C to 7.4 °C from October 2021 to January 2022. The total monthly rainfall was lowest in January 2022 (13.4 mm) and highest in December 2022 (178.8 mm) (Meteorological Service of New Zealand Ltd 2022).

3.2.1 Test-day records

Collections of test-day records started after the lambs were fully weaned. A total of 622 test-day records were gathered from 169 ewes between October 2021 and the end of January 2022 to obtain 2 to 4 milk tests from each ewe during the milk production season (Appendix 1 Table 5). The flock was dried off in mid-February.

Milk yields of individual animals were manually recorded from the volume taken from test buckets in the afternoon (at 2:30 pm) and on the following morning (at 6 am) in

October, when milking was happening twice a day (TAD). On the 1st of November 2021, the milking frequency was shifted to once a day (OAD) at the discretion of the farmer, happening in the afternoons only (at 2:30 pm). Reducing the milking frequency close to summer is a common practice on this farm to align with pasture availability. On each test day, a representative milk sample was taken from each animal after measuring milk yield.

Lambing dates ranged from the 26th of July to the 6th of November 2021, and the median lambing date was the 20th of August 2021. The deviation from the median lambing date of the flock was calculated for each ewe (ewe lambing date − median lambing date of the flock). Due to the shift to OAD milking in November, late-lambing ewes were not milked TAD in early lactation. To adjust for the milking frequency, days in TAD milking were calculated for each ewe (1st of November − weaning date). Information on litter sizes and the ages of animals were supplied to the study.

3.2.2 Milk composition

Milk samples were analysed by Milk Test NZ Ltd (Hamilton, NZ) using a Combi FOSS instrument (Foss Analytics, Hillerod, DK). The composition analysis included fat (%), protein (%), lactose (%) (ISO 9622), and somatic cell count (cells/mL) (ISO 13366-2). The yield of milk solid components (fat, protein, and lactose) on each test day was calculated by multiplying the milk volume by the concentration of milk solid components.

3.2.3 Pasture analyses

The flock had access to fresh white clover (Trifolium repens)/lucerne (Medicago sativa) pasture, and whole-grain maize and wheat were given as supplements during milking (approximately 600 g of supplement feed per milking). Samples of fresh pasture were taken for analysis on milk-sampling days by hand-plucking throughout the paddock where the animals were grazing the day before. These samples were freeze-dried and ground (Wiley mill) and analysed by the Nutrition Laboratory at Massey University (Palmerston North, NZ) using a near-infrared reflectance spectroscopy technique (Corson et al. 1999) using an MPA Analyser (Bruker Corporation, Billerica, Massachusetts, USA) to evaluate metabolisable energy (ME), crude protein (CP), neutral detergent fibre (NDF) content, and organic matter digestibility (OMD).

3.2.4 Modelling lactation curve

Records of milk, fat, protein, and lactose yields of all animals were plotted against time. Time was defined as d = days in milk − 35, as most records were made 35 days after the lambing date due to the exclusive suckling period. The shape of the curve of the plotted data was then examined.

Legendre polynomials were chosen to standardise values to the interval [-1, …., 1], and the coefficients were then calculated using the Rodrigues formula (Askey 2005):

$P0(t) = 1,$

$P1(t) = x,$

$P2(t) = \frac{1}{2}(3x^2 - 1),$

$P3(t) = \frac{1}{2}(5x^3 - 3x),$

$P4(t) = \frac{1}{8}(35x^4 - 30x^2+3),$

$P5(t) = \frac{1}{8}(63x^5 - 70x^3+15x)$

where $x = -1 + 2$, and $\frac{(t - tmin)}{(tmax - tmin)}$, with tmin = 1 and tmax = 130.

Day 1 corresponded to day 35 of lactation.

The random regression model was represented as follows:

$$y_{ti} = (\beta_0 P_0 + \beta_1 P_{1t} + \beta_2 P_{2t} + \cdots + \beta_n P_{nt}) + (\alpha_{0i} P_0 + \alpha_{1i} P_1 + \alpha_{2i} P_2 + \cdots + \alpha_{ni} P_n) + e_{ti},$$

where β values are the regression coefficients of the lactation curve of the population, α values are random regression coefficients describing the lactation curve for animal i, n is the maximum polynomial order, and e_{ti} is the random residual for animal i at time t. The estimates of β and α were obtained using the MIXED procedure of SAS version 9.4 (SAS 2004) with the COVTEST option for covariance parameter estimates. Polynomials of orders 2, 3, 4, and 5 were tested. Based on the Akaike (AIC) and Bayesian (BIC) information criteria (smallest is the best), an orthogonal polynomial of order 4 was considered the best fit for modelling daily milk and lactose yields. An orthogonal polynomial of order 5 was considered the best fit for modelling fat and protein daily yields.

The best covariance structure of random residuals was a diagonal-constant matrix (Toeplitz) for the modelling of repeated records on the same animal, also based on AIC and BIC values (Wolfinger 1993).

The somatic cell score (SCS) was calculated for each test-day record as SCS = Log_2(SCC). It was not possible to model the lactation curve for SCS, as the records did not follow any pattern throughout lactation. Thus, the average SCS was calculated for each lactation.

After choosing the best order of fit and the covariance structure for the models and computing the regressor coefficients (α0 to α4 or α0 to α5, depending on the trait) of each ewe, the daily milk yield was predicted from 35 to 164 days in milk (or from t = 1 to t = 130) using Legendre polynomial models of order 4 for milk and lactose yields and order 5 for fat and protein yields. Then, the predicted yields on each day of the lactation were summed to obtain an estimated total milk yield produced by each ewe for 130 days (from day 35 to day 164 of lactation). The measure of lactation persistency was defined as the estimated yield produced from day 101 to 164 divided by the estimated yield produced from day 35 to day 100 and expressed as a percentage. The higher this ratio, the higher the persistency. More persistent lactation will have a flatter curve, with the persistency proportion approaching one.

3.2.5 Measures of goodness of fit

The actual (A) and predicted (P) values for milk, fat, protein, and lactose yields were compared using linear regression of the actual on predicted values using PROG GLM in SAS version 9.4 software (SAS 2004) to obtain the slope and the mean square error (MSE). The relative prediction error (RPE) was calculated as the square root of MSE divided by the mean of the actual values (μ_A), multiplied by 100, as per the following equation (Rook et al. 1990):

$$\text{RPE} = \frac{\sqrt{\text{MSE}}}{\mu_A} \times 100.$$

The correlation coefficient indicates the closeness of actual and predicted values and was obtained using PROC CORR in SAS version 9.4 software (SAS 2004). To evaluate the agreement between paired readings, the concordance correlation coefficient between predicted and actual values was calculated as follows (McKusick et al. 2001):

$$\rho_{ccc} = \frac{2\sigma_{PA}}{\sigma_P^2+\sigma_A^2+(\mu_P-\mu_A)^2},$$

where σ_{PA} is the covariance, σ_P^2 and σ_A^2 are the variances, μ_P and μ_A are the means, and P and A refer to predicted and actual values.

3.2.6 Statistical analysis

All statistical analyses were performed using the statistical package SAS version 9.4 (SAS 2004). Descriptive statistics (mean, standard deviation, minimum and maximum values, and coefficient of variation) for total yields were obtained with the MEAN procedure. Analysis of variances for the estimated total yields and regression coefficients were performed using the MIXED procedure with a linear model that included the fixed effects of ewe coat colour (i = categorical variable with two levels: black or white) as an indicator of animal variety within the breed, litter size (categorical variable with two levels: j = 1 lamb or 2 lambs and greater), and ewe age class or parity number (categorical variable with four levels: k = 1, 2, 3, and 4 years and older), with days in TAD milking as a covariate (β_1). The covariate days in TAD was used in the model instead of the deviation from the median lambing date, as these traits were strongly correlated (−0.6). The model is represented as follows:

$$y_{ijkl} = \mu + \text{coat colour}_i + \text{litter size}_j + \text{age}_k + \beta_1 \text{days in TAD}_{ijkl} + e_{ijkl}$$

where y_{ijkl} represents the dependent variables, which include the estimated total yields of milk, fat, protein, and lactose, the regression coefficients of individual animals, and lactation persistency. Least squares means for each class of fixed effects and standard errors were obtained and used for mean comparisons using Fisher's least significant difference test.

3.3 Results

3.3.1 Pasture quality

The level of metabolisable energy (ME) in the pasture dry matter was high (12 MJ/kg) in early October and dropped throughout the season to 8.2 MJ/kg in late January (Table 3.1). The level of crude protein in the pasture dry matter also dropped from 26.7 to 13.5%DM

throughout the season, and the levels of non-detergent fibre increased from 36.5 to 60.6%DM. Consequently, organic matter digestibility decreased from >84 to only 57.6%.

Table 3.1. Pasture analyses using the near-infrared reflectance spectroscopy technique based on dry matter[1].

	DM	ME	CP	NDF	OMD
Month	(%)	MJ/kg DM	% DM	% DM	%
October	19.1	12.0	26.7	36.5	>84.0
November	19.9	11.4	17.3	45.9	77.2
December	24.7	10.5	13.5	49.2	72.5
January	29.7	8.2	14.1	60.6	57.6

[1]DM = dry matter; ME = metabolisable energy; CP = crude protein; NDF = non-detergent fibre; OMD = organic matter digestibility.

3.3.2 Average production

The mean lactation length of the flock in the present study was 165 days, and the mean milking length was 108 days. The flock produced an average of 86.1 kg of total milk yield per ewe (0.7 kg per ewe/day) and an average of 5.1 kg of total fat, 4.5 kg of total protein, and 4.1 kg of total lactose yield per ewe for 130 days of milking (Table 3.2).

Table 3.2. Descriptive statistics of production variables considered in the genetic evaluation of Dairymeade sheep in 2021–2022 season (n = 169 lactations).

Trait	Mean	SD[1]	Min	Max	CV[2] (%)
Lactation length[3] (days)	165	23.6	90	203	14
Milking length[4] (days)	108	25.8	35	133	24
Lactation yields[5] (kg)					
Milk	86.1	26.1	25.1	168.8	30
Fat	5.1	1.4	2.0	9.6	28
Protein	4.5	1.2	1.7	8.2	27
Lactose	4.1	1.3	1.2	8.0	31
Persistency[6] (%)					
Milk	55.8	8.9	35.9	94.9	16

Fat	72.7	11.1	53.5	115.5	15
Protein	65.7	9.5	46.2	99.2	15
Lactose	52.3	8.4	32.2	86.8	16
Average SCS[7]	16.9	1.7	14.7	23.6	10
Age (years)	2.8	1.1	1	4	39
Litter size	1.7	0.6	1	3	33

[1]SD = standard deviation.
[2]CV = coefficient of variation.
[3]Lactation length (from lambing to dry-off).
[4]Milking length (from weaning to dry-off).
[5]Lactation yields estimated from daily yields from 35 to 164 days of lactation.
[6]Lactation persistency defined as yield from day 101 to 164 divided by yield from day 35 to 100, expressed as percentage.
[7]Average of SCS, calculated as SCS = Log_2(somatic cell count).

3.3.3 Model adequacy

The measures of goodness of fit are presented in Table 3.3. The intercepts of the regression lines of the actual on predicted values were negative, but not departing significantly from zero, and the slopes were all greater than 1.0, with relative predicted error close to 10%. The correlation and concordance correlation coefficients were close to one.

Table 3.3. Measures of goodness of fit of the model of the lactation curves for milk and lactose using random regression with a fourth-order Legendre polynomial and for fat and protein using random regression with a fifth-order Legendre polynomial.

	Regression Line of A on P				
	a	b	RPE (%)	r	ρ_{ccc}
Milk (kg/day)	−0.03 ± 0.01	1.05 ± 0.01	11.65	0.975	0.969
Fat (g/day)	−4.23 ± 0.53	1.10 ± 0.01	12.67	0.962	0.952
Protein (g/day)	−2.65 ± 0.36	1.07 ± 0.01	9.92	0.977	0.972
Lactose (g/day)	−1.44 ± 0.37	1.04 ± 0.01	12.79	0.972	0.969

a and b are the intercept and slope of the regression line of the actual (A) on predicted (P) values; RPE = relative predicted error; r = correlation coefficient; ρ_{ccc}= concordance correlation coefficient.

3.3.4 Factors affecting the lactation curves

The F- and p-values for fixed effects are presented in Table 3.4. Least squares means for lactation yields and for lactation persistency for the different class effects are presented in Tables 3.5 and 3.6. The least squares means for the regression coefficients are provided in Appendix 1 Tables 1, 2, 3, and 4). The effects of age (parity number) and days in TAD milking were significant on all total yields (Table 3.4). The factor that had the greatest effect on the total yields was days in TAD milking (largest F-values).

One-year-old ewes produced significantly less milk, fat, protein, and lactose than older ewes (Table 3.5). Three-year-old ewes produced the highest yields of milk, protein, and lactose, whereas four-year-old ewes produced the highest yield of fat. Ewes that lambed late in relation to the median lambing date of the flock missed TAD milking in early lactation and produced significantly less milk, fat, protein, and lactose than early-lambing ewes that were milked TAD in early lactation, with the largest F-value for the effect of days in TAD on total fat yield (27.47).

Ewe age affected the lactation persistency of milk and lactose yields, litter size affected the lactation persistency of protein yield, and milking frequency strongly affected the lactation persistency of fat yield with a large F-value (10.96). Late-lambing ewes that were not milked TAD in early lactation had a significantly higher lactation persistency of fat than early-lambing ewes (Table 3.6). Coat colour had no significant effect on any trait. None of the factors significantly affected the somatic cell score.

Table 3.4. F-values for effects of ewe coat colour, ewe age, litter size (LS), and days in twice-a-day (TAD) milking on milk production traits in Dairymeade sheep in the production season of 2021–2022.

Trait	Coat colour	Age	LS	Days in TAD
Lactation yields[1] (kg)				
Milk	0.07	6.70 ***	0.63	19.86 ***
Fat	0.61	3.83 **	0.00	27.47 ***
Protein	0.01	4.38 **	1.12	20.51 ***
Lactose	0.03	6.71 ***	0.63	17.48 ***
Persistency[2] (%)				

Milk	1.90	3.55 *	1.96	0.00
Fat	1.29	1.55	1.69	10.96 ***
Protein	0.50	1.37	5.09*	2.03
Lactose	1.33	3.34 *	1.46	0.45
Average SCC	0.06	0.51	0.09	0.01
Average SCS[3]	0.94	1.70	1.48	0.79

[1]Lactation yields estimated from daily yields from 35 to 164 days of lactation. [2]Lactation persistency defined as yield from day 101 to 164 divided by yield from day 35 to day 100, expressed as percentage. [3]Average of SCS, calculated as SCS = Log_2(somatic cell count). Statistical significance is given as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3.5. Least squares means (Mean) and standard errors (SE) of lactation yields of milk (kg), fat (kg), protein (kg), and lactose (kg) estimated for 130 days after weaning for different ewe ages (year), litter sizes, coat colours, and days in twice-a-day (TAD) milking at Kingsmeade farm during the production season 2021–2022.

		Milk		Fat		Protein		Lactose	
Effect	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age									
1	26	64.3[b]	5.4	4.1[b]	0.30	3.6[b]	0.30	3.1[b]	0.30
2	47	85.8[a]	3.6	5.0[a]	0.20	4.5[a]	0.17	4.2[a]	0.18
3	39	92.1[a]	3.6	5.2[a]	0.20	4.7[a]	0.17	4.4[a]	0.18
≥4	57	90.2[a]	3.0	5.3[a]	0.17	4.6[a]	0.14	4.3[a]	0.15
Litter size									
1	66	81.7	2.8	4.9	0.16	4.3	0.14	3.9	0.14
2	103	84.5	2.9	4.9	0.16	4.4	0.14	4.1	0.14
Coat colour									
Black	33	82.5	4.0	4.8	0.22	4.3	0.20	3.9	0.20
White	136	83.7	1.9	5.0	0.11	4.4	0.09	4.0	0.10
Days in TAD									
0	57	71.2[b]	3.2	4.1[b]	0.18	3.8[c]	0.15	3.4[b]	0.16
14	36	86.8[a]	4.0	5.1[a]	0.22	4.5[b]	0.19	4.2[a]	0.20

21	38	86.3[a]	4.0	5.3[a]	0.22	4.4[b]	0.19	4.2[a]	0.20
28	38	94.8[a]	4.2	5.6[a]	0.24	5.0[a]	0.20	4.5[a]	0.21

n = number of ewes within each category. [a,b,c] Least squares means with different superscripts within effect are significantly different ($p < 0.05$).

Table 3.6. Least squares means (Mean) and standard errors (SE) of lactation persistency (%)[1] of milk, fat, protein, and lactose yields, for different ewe ages, litter sizes, coat colours, and days in twice-a-day (TAD) milking at Kingsmeade farm during 2021–2022 season.

		Milk		Fat		Protein		Lactose	
Effect	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age									
1	26	61.5[a]	2.2	78.3	2.6	69.9	2.3	57.6[a]	2.1
2	47	53.7[b]	1.4	74.1	1.7	65.1	1.5	50.5[b]	1.4
3	39	54.5[b]	1.4	71.8	1.7	64.6	1.5	51.1[b]	1.4
>4	57	54.2[b]	1.2	72.8	1.4	65.0	1.3	50.9[b]	1.2
Litter size									
1	66	57.0	1.1	75.4	1.4	67.9[a]	1.2	53.4	1.1
2	103	55.0	1.2	73.1	1.3	64.4[b]	1.2	51.7	1.1
Coat colour									
Black	33	54.8	1.6	75.4	1.9	65.5	1.7	51.5	1.5
White	136	57.2	0.8	73.1	0.9	66.9	0.8	53.5	0.7
Days in TAD									
0	57	55.7	1.2	78.6[a]	1.5	67.1	1.4	51.3	1.2
14	36	56.3	1.6	74.2[ac]	1.9	67.1	1.7	53.0	1.5
21	38	57.2	1.6	70.7[bc]	1.9	66.6	1.7	54.3	1.5

28	38	55.0	1.7	71.4^{bc}	2.0	63.6	1.8	52.0	1.6

n = number of ewes within each category. [1] Lactation persistency (%) = (yield from day 101 to 164 / yield from day 35 to 100) × 100. [a,b,c] Least squares means with different superscripts within effect are significantly different ($p < 0.05$).

3.5 Lactation curves

Lactation curves for daily yields of milk, fat, protein, and lactose for different ewe ages are presented in Figure 3.1. Overall, milk, fat, protein, and lactose yields declined over the course of lactation (from 35 to 164 days in milk), and no initial peak was observed. This descending trend is not only a physiological occurrence, but it is also likely to be a result of reduced pasture quantity and quality in this seasonal pasture-based system. An atypical increase towards the end of the season (between 100 and 150 days in milk) was observed in the lactation curves for different ewe ages, mainly for fat and protein yields (Figure 3.1 B, C). Lactation curves for one-year-old ewes were visually distinct from the lactation curves of two-, three-, and four-year and older ewes. Overall, milk yield increased with age (parity), with 3-year-old ewes producing the highest yields, which then descended afterward, producing more than 4-year-old ewes.

3.4 Discussion

3.4.1 Flock performance

There are scarce scientific publications on the milk production of dairy sheep in New Zealand to enable comparisons, but the low daily production per ewe in this flock is noticeable. The low total yield achieved per ewe can be attributed to the long exclusive suckling period, which lasted, on average, 57 days. In addition, the rapid decline in milk production was largely influenced by reduced pasture quality in this seasonal pasture-based system. The composition percentage was within the range reported previously for East Friesians (Schaeffer 2004; McKusick 2001). However, due to the lower total milk yield, this flock also produced low total yields of fat, protein, and lactose compared to other studies in New Zealand and overseas (McMillan et al. 2014; Scholtens et al. 2018; McKusick et al. 2001; Hamann et al. 2004).

Although higher or similar yields have been reported for other New Zealand flocks, those studies had a short lamb suckling period (Gosling et al 1997; McMillan et al 2014;

Scholtens et al 2018) or were in a mixed regime of suckling and milk collection (Scholtens et al 2018). Gosling et al (1997) reported 116 L over 147 days and higher solids for New Zealand Dorset ewes that lambed in spring, with lambs removed at four days of age. The present flock produced a similar total milk yield to those reported for Poll Dorset ewes (86.8 L/ewe) and East Friesian crossed ewes (113.1 L/ewe) milked for 102 days, but their lambs were artificially reared (Newman et al 1999). McMillan et al (2014) reported a much higher milk yield (310 L of milk produced over 147 days) in East Friesian crossed ewes and hoggets in a New Zealand pasture-based system, with no suckling period. More recently, Scholtens et al (2018) reported 234 kg for 126 days of lactation in New Zealand East Friesian crossed ewes.

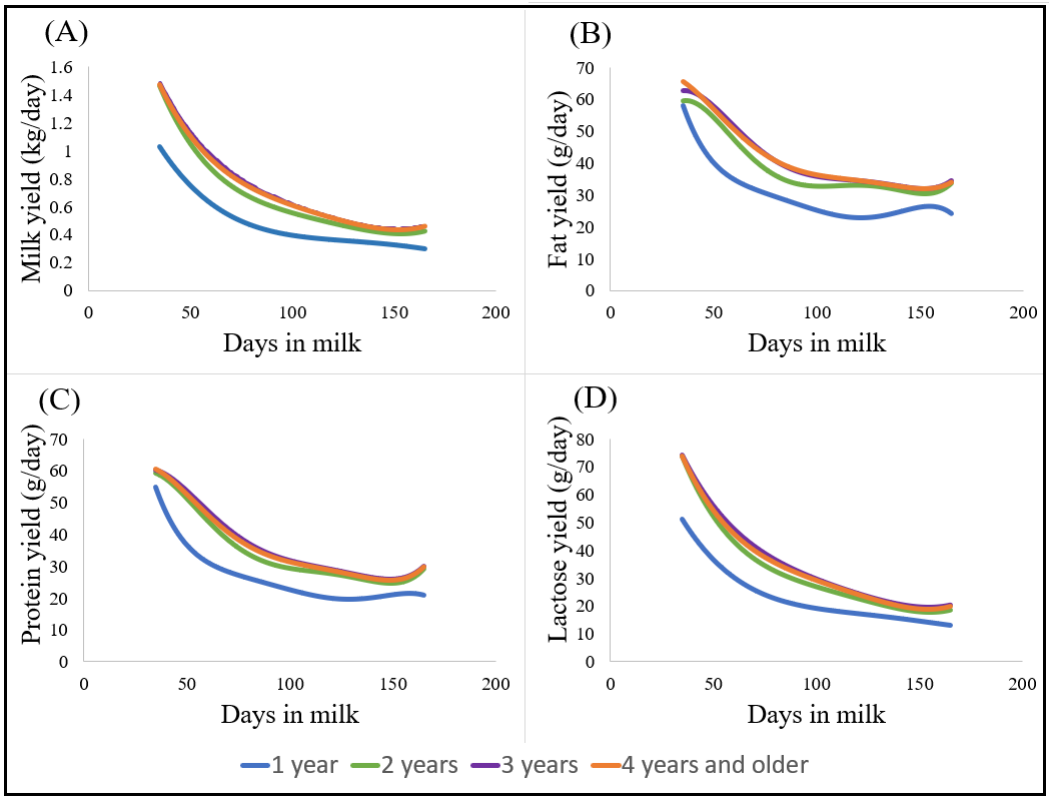


Figure 3.1. Lactation curves for daily yields of milk (A), fat (B), protein (C), and lactose (D) in lactations of Dairymeade ewes of one (blue), two (green), three (purple), and four years old or older (orange), modelled using orthogonal polynomials of order 4 for milk and lactose yields and of order 5 for fat and protein yields.

The low daily production per ewe achieved in this extensive farming system can also be attributed to low forage availability in the summer caused by hot and dry weather

conditions. The use of a long exclusive suckling period compromised the total milk yield per ewe as the initial peak of milk production was missed. It has been suggested that a full suckling period during the first 30 or 60 days of lactation can lower yields by 20–25% or 45–50%, respectively (McMillan et al 2014). It is important to mention, however, that multiple factors are involved in a farmer's choice of a weaning system, and these are based not only on the total yield produced but also on the cost, work, stress, and net benefit.

In addition, the breed used in the present study is mainly founded from East Friesians, which was the first dairy breed of sheep to be imported to New Zealand in the 1990s and provided an opportunity for increasing milk yield in sheep dairying (Newman et al 1999; Allison 1996). Selection based upon temperament, health, and lactation length has been in place at this farm since 1996 and contributed to the establishment and improvement of the Dairymeade breed. However, East Friesians are known for being a high-yielding dairy breed originally selected for intensively managed systems and may have limited production on extensive systems. Under hot or dry conditions, East Friesian ewes have been noted to produce low to moderate yields, and poor adaptability to the Mediterranean climate and semi-arid conditions has been reported (Gootwine et al 1996; Angeles-Hernandez et al 2014). East Friesians managed in intensive or mixed indoor–outdoor systems can express their full genetic potential for milk production, and high milk yields of over 400 kg per lactation have been reported (Hamann et al 2004).

The persistency of fat yield was the highest, followed by protein, milk, and lactose yields, agreeing with the findings of Jonas et al. (2011). Better conditions for pasture growth in the middle towards the end of lactation (December) are likely to have affected the persistency of fat and protein yields obtained in the present study, as these traits are largely influenced by the level of feeding (Pulina et al 2006). Although there is no single reference method for the calculation of lactation persistency (Sölkner and Fuchs 1987; Swalve and Gengler 1999; Grossman et al 1999), more persistent lactation will have a flatter curve, with the persistency proportion approaching 100%. The milk yield persistency of this flock was lower and fat and protein yield persistency was higher than that reported by Scatà et al. (2010) using a similar method for calculation.

3.4.2 Model adequacy and lactation curves

All measures of goodness of fit presented in Table 3.3 indicate that random regression with Legendre polynomials is an adequate technique to model the lactation curves of dairy sheep in this commercial flock. The estimates of the intercepts were close to zero, and the slopes were all slightly greater than 1.0, meaning that the models tended to over-predict at low actual values and under-predict at high actual values, creating RPEs greater than 10%. According to Fuentes-Pila (1996), an RPE <10% is considered satisfactory, and between 10 and 20% is relatively good for prediction models. However, Lin's concordance correlations (Lin 1989) close to one indicate that the actual and predicted values were in high agreement with low biases in the mean and regression line of the actual on predicted values.

The typical lactation curve is represented by a rapid increase to a peak in the first few weeks of lactation before gradually declining until the end of lactation. This pattern has been reported in various breeds of sheep (Oravcová et al. 2006; Hunter et al. 2015). The farm used in this study has an exclusive suckling period when there is no milk collection, meaning that few milk yield records were taken in the very early stages of lactation, and this initial peak was not observed for most of the individual lactation curves.

Overall, milk yield, fat, protein, and lactose yields declined as lactation progressed from day 35 to 164. An atypical small increase and stabilisation at the end of lactation were observed in the lactation curves of fat and protein yields of ewes of 2 years or older (Figure 3.1 B, C), being more obvious for fat yield, as the fat content is the most variable component in milk, and changes are more pronounced with the feeding level (Pulina et al. 2006).

Others have reported "atypical" lactation curves defined as continuously decreased milk production without a lactation peak, even when sampling was performed in the first week of lactation (Angeles-Hernandez et al. 2013). In pasture-based systems, animals that have low forage availability and low supplementation are not able to fully express their productive potential (Padel 2000) and the peak may not be observed. The peak is also not observed in less selected animals (Peralta-Lailson et al. 2005). In grazing systems, weather affects not only pasture availability but also comfort and stress in animals, therefore also playing an important role in the shape of lactation curves (Abecia et al. 2020).

The lactation curves obtained in this study had a moderate persistency, and a rapid rate of decline in milk production was observed. In theory, an ideal lactation curve for greater milk yield production would have a high peak and a moderately flat trend afterwards. However, correlations between peak yield and persistency have been reported to be negative in cows (Hickson et al. 2006) and in sheep (Velarde-Guillén et al. 2022). Additionally, peak yield has been reported to be more correlated with a high lactation yield than with lactation persistency (Pollott 2000).

On the other hand, a very high peak yield has been associated with a highly negative energy balance and metabolic stress in early lactation, and sheep tend to reduce their milk production more markedly than cows when in a negative energy balance (Cannas et al. 2002). In addition, flatter curves have been related to better animal health and a reduction in feeding costs (Sölkner and Fuchs 1987; Scatà et al. 2010; Dekkers et al. 1998). Therefore, the peak and persistency should be carefully considered when selecting dairy animals for milk production. Furthermore, feed management decisions during lactation are likely to mask the real persistency (Swalve and Gengler 1999).

3.4.3 Animal factors

Milk yield increased with age (parity), but 3-year-old ewes produced more than 4-year-old ewes. The parity number has been widely reported to significantly affect milk production (Velarde-Guillén et al. 2022; Pollott & Gootwine 2004; Angeles-Hernandez et al. 2018; Robles Jimenez et al. 2020). The mammary glands of primiparous ewes are still not fully developed, and therefore, they have a less pronounced peak yield and are flatter in shape (Abdelsayed et al. 2014).

One-year-old ewes produced 20.9, 0.9, 0.9, and 1.1 kg less milk, fat, protein, and lactose yields, respectively, than 3-year-old ewes. Three-year-old ewes produced more than 4-year-old ewes. However, one-year-old ewes had higher lactation persistency than older ewes, agreeing with other published results (Pollott & Gootwine 2004; Carta et al. 1995). One-year-old ewes' lactation persistency of milk and lactose was 7.8% and 7.1% higher than that of two-year-old ewes, and the effect was significant.

Notably, from a simple analysis of Pearson correlations between effect variables, age (parity) was positively correlated with litter size (0.27) and with days in TAD milking

(0.34). This means that older ewes tended to give birth to multiple lambs and to lamb earlier than primiparous ewes. Litter size is reportedly known to be smaller for primiparous ewes compared to multiparous ewes (Pollott & Gootwine 2004; Annett & Carson 2006). The mating management of the farm, where ewe hoggets are mated later than mature ewes, is likely to be the main determinant of the lambing date in this flock. However, it is also known that mature ewes tend to get pregnant faster than young ewes in the mating season and therefore also lamb earlier in the lambing season. Young ewes can also display shorter and less intense oestrus period (Edey et al. 1978) and need to be served by the ram on at least three occasions (Allison 1982).

There was no difference ($p > 0.05$) in milk yield between ewes with twin lambs and ewes with a single lamb. However, there was a trend for ewes that lambed twins to produce about 2.8 kg more milk than equivalent ewes with a single lamb. Other studies have shown that litter size significantly affects milk production, in that ewes with twins or triplets yield more milk than single-lambing ewes (Angeles-Hernandez et al. 2013; Robles Jimenez et al. 2020). In the current study, ewes with twins produced the same fat yield and slightly higher protein and lactose yields than ewes with a single lamb, though this was also not significant. Others have shown the contents of fat and protein to significantly vary with the effect of litter size (Oravcová et al. 2007).

Ewes that carry twins are expected to produce more milk because of their higher secretion of placental lactogen due to a higher placental mass, stimulating the greater development of the mammary gland (Butler et al. 1981). Additionally, the higher stimulus of the mammary gland during suckling by lambs stimulates the higher production of milk (Negrão et al. 2001). However, it has been suggested that this is observed in the first stage of lactation, mainly until the peak yield, showing no significant differences after the 10th–17th week of lactation (Angeles-Hernandez et al. 2013; Gabiña et al. 1993). In the current study, few records were made in the very early stage of lactation due to the suckling period, and the lactation peak was not observed; therefore, differences in milk production due to litter size were not pronounced.

Interestingly, litter size did not significantly affect the lactation persistency of milk, fat, or lactose yields but affected the lactation persistency of protein yield, even though litter size had no significant effect on protein yield. Ewes that had twin lambs had a sharper decline in protein yield during lactation compared with ewes that had a single lamb. Previous

studies have shown a small litter size to be correlated with a lower total milk yield and with flatter lactation curves (Pollott & Gootwine 2004; Kominakis et al. 2002).

In this study, coat colour did not significantly affect milk production. However, there was a trend for white ewes to produce 1.2 kg more milk yield than ewes with black colouring (Table 3.5). Only 24% of the flock were black-coated due to the white colour being dominant over black/brown coats in various breeds of sheep (Koseniuk et al. 2018). Other studies have shown the colour/variety to be associated with production traits in other sheep breeds (Peralta-Lailson et al. 2005; Angeles-Hernandez et al. 2018; Pascual-Alonso et al. 2014), indicating the effect of the genotype on production. In the study by Peralta-Lailson et al. (2005), the variety significantly affected the milk yield of Creole sheep in Mexico, with brown ewes producing more than white and black ewes, which was attributed to better lactation persistency.

The shift in the milking frequency of the flock from TAD to OAD in November strongly affected milk production. Ewes that lambed late missed the TAD milking period, producing 15.7, 1.0, 0.7, and 0.8 kg less milk, fat, protein, and lactose, respectively, compared to ewes that were milked for 14 days TAD in early lactation. Additionally, the lambing date itself can affect the milk production of ewes (Hickson et al. 2006; Dekkers et al. 1998). In the present study, the lambing date and days in TAD milking were correlated and were confounding effects if simultaneously included.

The strong effect of a TAD milking frequency in early lactation on fat yield affected the lactation persistency of fat. Ewes that were milked TAD in the first part of lactation produced a higher yield of fat in this period, and therefore, production declined more sharply after shifting to OAD, translating into worse lactation persistency compared to ewes that were milked OAD from the start. Other studies have confirmed a significant decline in milk production with the reduction from twice- to once-a-day milking (Nudda et al. 2002; Koutsouli et al. 2017).

3.5 Conclusions

There are a limited number of scientific publications on pasture-based dairy sheep production in New Zealand. The differences in milk production between the current study and other studies were not only attributed to the different genetic makeup of the animals

but also due to the different environment, feeding, and lamb weaning systems. It is also important to note that due to the long exclusive lamb suckling period of the farm, this study only modelled the after-peak curves and not the full lactation curves. The low total yield produced is also a consequence of the sharp decrease in the lactation curves, which were largely influenced by the reduced pasture quality in this seasonal pasture-based system. This study provides experiences for a larger-scale animal evaluation and breeding programme to improve dairy sheep genetics for New Zealand farming systems.

References

Abdelsayed M, Thomson PC, Raadsma HW. 2014. A review of the genetic and non-genetic factors affecting extended lactation in pasture-based dairy systems. Animal Production Science. 55: 949–966.

Abecia JA, Garcia A, Castillo L, Palacios C. 2020. The effects of weather on milk production in dairy sheep vary by month of lambing and lactation phase. Journal of Animal Behavior and Biometeorology. 5: 56–63.

Allison AJ. 1982. Techniques of modifying reproductive performance. In: Wickham GA, McDonald MF, editors. Sheep Production, Volume 1—Breeding and Reproduction. New Zealand Institute of Agricultural Science, Ray Richards Publisher: Wellington, New Zealand. p. 239–263.

Allison AJ. 1996. More opportunities with sheep: The East Friesian Breed and its relevance to the New Zealand sheep industry. Society of Sheep, Beef Cattle Veterinarians of New Zealand Veterinary Association. 26: 18–30.

Angeles-Hernandez JC, Albarran-Portillo B, Gonzalez AG, Salas NP, Gonzalez-Ronquillo M. 2013. Comparison of mathematical models applied to F1 dairy sheep lactations in organic farm and environmental factors affecting lactation curve parameters. Asian-Australasian Journal of Animal Sciences. 26: 1119–1126.

Angeles-Hernandez JC, Ortega OAC, Perez AHR, Gonzalez-Ronquillo M. 2014. Effects of crossbreeding on milk production and composition in dairy sheep under organic management. Animal Production Science. 54: 1641–1645.

Angeles-Hernandez JC, Radic Schilling S, Vera-Arias MA, Echeverria-Perez RA, Castelán-Ortega OA, Ramirez Perez AH, Gonzalez Ronquillo M. 2018. Effect of live weight pre-and post-lambing on milk production of East Friesian sheep. Italian Journal of Animal Science. 17: 184–194.

Annett RW, Carson AF. 2006. Effects of plane of nutrition during the first month of pregnancy on conception rate, foetal development and lamb output of mature and adolescent ewes. Animal Science. 82: 947–954.

Askey R. 2005. The 1839 paper on permutations: Its relation to the Rodrigues formula and further developments. In Mathematics and Social Utopias in France: Olinde Rodrigues and His Times, History of Mathematics; Altmann S, L., Ortiz EL, Eds.; American Mathematical Society: Providence, RI, USA. 28: 105–118. ISBN 978-0-8218-3860-0.

Balthazar CF, Pimentel TC, Ferrão LL, Almada CN, Santillo A, Albenzio M, Mollakhalili N, Mortazavian AM, Nascimento JS, Silva MC, et al. 2017. Sheep milk:

Physicochemical characteristics and relevance for functional food development. Comprehensive Reviews in Food Science and Food Safety. 16: 247–262..

Bencini R, Pulina G. 1997. The quality of sheep milk: A review. Australian Journal of Experimental Agriculture. 37: 485–504.

Bliss AE, Chylinski C, Luo D, Schütz KEE, Lowe K, Peterson SW, Stevens D, MacDonald T, McCoard SA. 2018. The impact of different management systems on behaviour and milk production of dairy ewes in different stages of lactation; a case study. New Zealand Journal of Animal Science and Production. 78: 116–121.

Butler W, Fullenkamp S, Cappiello L, Handwerger S. 1981. The relationship between breed and litter size in sheep and maternal serum concentrations of placental lactogen, estradiol and progesterone. Journal of Animal Science. 53: 1077–1081.

Cannas A, Nudda A, Giuseppe P. 2002. Nutritional strategies to improve lactation persistency in dairy ewes. In Proceedings of the 8th Great Lakes Dairy Sheep Symposium Cornell University, Ithaca, NY, USA, 7–9 November 2002; pp. 17–59.

Carta A, Sanna SR, Casu S. 1995. Estimating lactation curves and seasonal effects for milk, fat and protein in Sarda dairy sheep with a test day model. Livestock Production Science. 44: 37–44.

Corson DC, Waghorn GC, Ulyatt MJ, Lee J. 1999. NIRS: Forage analysis and livestock feeding. Proceedings of the New Zealand Grassland Association. 61: 127–132.

Danell B. 1982. Studies on lactation yield and individual test-day yields of Swedish dairy cows. III. Persistency of milk yield and its correlation with lactation yield. Acta Agriculturae Scandinavica. 32: 93–101.

Dekkers JCM, Ten Hag JH, Weersink A. 1998. Economic aspects of persistency of lactation in dairy cattle. Livestock Production Science. 53: 237–252.

Downie-Melrose KS. 2014. A bio-economic feasibility study of dairy sheep systems in Canterbury. Lincoln University: Christchurch, New Zealand. Available online: https://researcharchive.lincoln.ac.nz/handle/10182/6423 (accessed on 8 December 2022).

Edey TN, Kilgour R, Bremner K. 1978. Sexual behaviour and reproductive performance of ewe lambs at and after puberty. Journal of Agricultural Science. 90: 83–91.

Fuentes-Pila J, DeLorenzo MA, Beede DK, Staples CR, Holter JB. 2006. Evaluation of equations based on animal factors to predict intake of lactating Holstein cows. Journal of Dairy Science. 79: 1562–1571.

Gabiña D, Arrese F, Arranz J, Beltran De Heredia I. 1993. Average milk yields and environmental effects on latxa sheep. Journal of Dairy Science. 76: 1191–1198.

Geenty KG, Davison PG. 1982. Influence of weaning age, milking frequency, and udder stimulation on dairy milk production and post-partum oestrus interval of Dorset ewes. New Zealand Journal of Experimental Agriculture. 10: 1–5.

Gootwine E, Goot H. 1996. Lamb and milk production of Awassi and East Friesian sheep and their crosses under a Mediterranean environment. Small Ruminant Research. 20: 255–260.

Gosling I, Knight T, Newman SA. 1997. Effects of season of lambing, stage of lactation and ewe age on milk volume and composition of machine-milked Dorset ewes. Proceedings of the New Zealand Society of Animal Production. 57: 212–217.

Grossman M, Hartz SM, Koops WJ. 1999. Persistency of lactation yield: A novel approach. Journal of Dairy Science. 82: 2192–2197.

Hamann H, Horstick A, Wessels A, Distl O. 2004. Estimation of genetic parameters for test day milk production, somatic cell score and litter size at birth in East Friesian ewes. Livestock Production Science. 87: 153–160.

Hickson RE, Lopez-Villalobos N, Dalley DE, Clark DA, Holmes CW. 2006. Yields and persistency of lactation in Friesian and Jersey cows milked once daily. Journal of Dairy Science. 89: 2017–2024.

Hunter T, Suster D, DiGiacomo K, Dunshea F, Cummins L, Egan A, Leury B. 2015. Milk production and body composition of single-bearing East Friesian × Romney and Border Leicester × Merino ewes. Small Ruminant Research. 131: 123–129.

ISO 13366-2 (IDF 148-2:2006). 2006. Milk—Enumeration of Somatic Cells—Part 2: Guidance on the Operation of Fluoro-Optoelectronic Counters. ISO: Geneva, Switzerland.

ISO 9622 (IDF 141:2013). 2013. Milk and Liquid Milk Products—Guidelines for the Application of Mid-Infrared Spectrometry. ISO: Geneva, Switzerland.

Jonas E, Thomson PC, Hall EJS, McGill D, Lam MK, Raadsma HW. 2011. Mapping quantitative trait loci (QTL) in sheep. IV. Analysis of lactation persistency and extended lactation traits in sheep. Genetics Selection Evolution. 43: 22–32.

Kirkpatrick M, Lofsvold D, Bulmer M. 1990. Analysis of the inheritance, selection and evolution of growth trajectories. Genetics. 124: 979–993.

Kominakis A, Volanis M, Rogdakis E. 2001. Genetic modelling of test day records in dairy sheep using orthogonal Legendre polynomials. Small Ruminant Research. 39: 209–217.

Kominakis AP, Rogdakis E, Koutsotolis K. 2002. Genetic aspects of persistency of milk yield in Boutsico dairy sheep. Asian-Australasian Journal of Animal Sciences. 15: 315–320.

Koseniuk A, Ropka-Molik K, Rubiś D, Smołucha G. 2018. Genetic background of coat colour in sheep. Archives of Animal Breeding. 61: 173–178.

Koutsouli P, Simitzis P, Theodorou G, Massouras Th, Bizelis I, Politis I. 2017. The effect of milking frequency reduction from twice to once daily on mammary physiology and animal welfare of two dairy Greek sheep breeds. Small Ruminant Research. 147: 18–24.

Kuchtík J, Sustova K, Urban T, Zapletal D. 2008. Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. Czech Journal of Animal Science. 53: 55–63.

Lees N, Lees I. 2018. Competitive advantage through responsible innovation in New Zealand's sheep dairy industry. International Food and Agribusiness Management Review. 21: 505–524.

Lin LIK. 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics. 45: 255–268.

Lopez-Villalobos N, King M, King J, Morris RS. 2017. Estimates of genetic parameters for direct and maternal genetic effects on weaning weights in Dairymeade sheep. Proceedings of the New Zealand Society of Animal Production. 77:129–132.

Macciotta NPP, Vicario D, Cappio-Borlino A. 2005. Detection of different shapes of lactation curve for milk yield in dairy cattle by empirical mathematical models. Journal of Dairy Science. 88: 1178–1191.

McKusick BC, Thomas DL, Berger YM. 2001. Effect of weaning system on commercial milk production and lamb growth of East Friesian dairy sheep. Journal of Dairy Science. 84: 1660–1668.

McMillan WH, McLachlan SJ, Hercus IS. 2014. Brief communication: High milk production in milked sheep grazed in large flocks in New Zealand. Proceedings of the New Zealand Society of Animal Production. 74: 52–54.

Meteorological Service of New Zealand Ltd. 2022. Available online: https://www.metservice.com/towns-cities/locations/masterton/past-weather (accessed on 5 September 2022).

Negrão JA, Marnet PG, Labussière J. 2001. Effect of milking frequency on oxytocin release and milk production in dairy ewes. Small Ruminant Research. 39: 181–187.

Newman S, Stieffel W. 1999. Milking performance of East Friesian Poll Dorset cross ewe hoggets. Proceedings of the New Zealand Society of Animal Production. 59: 125–128.

Nudda A, Bencini R, Mijatovic S, Pulina G. 2002. The yield and composition of milk in sarda, awassi, and merino sheep milked unilaterally at different frequencies. Journal of Dairy Science. 85: 2879–2884.

O'Connor HD. 2022. Government supports innovative dairy sheep sector to scale up. Available online: https://www.beehive.govt.nz/release/government-supports-innovative-dairy-sheep-sector-scale (accessed on 8 December 2022).

Oravcová M, Margetín M, Peskovicova D, Dano J, Milerski M, Hetényi L, Polák P. 2006. Factors affecting milk yield and ewe's lactation curves estimated with test-day models. Czech Journal of Animal Science. 51: 483–490.

Oravcová M, Margetín M, Peskovicova D, Dano J, Milerski M, Hetényi L, Polák P. 2007. Factors affecting ewe's milk fat and protein content and relationships between milk yield and milk components. Czech Journal of Animal Science. 52: 189–198.

Padel S. 2000. Strategies of organic milk production. Human-animal relationships: Stockmanship and Housing in Organic Livestock Systems. In Proceedings of the 3rd NAHWOA Workshop, Clermont-Ferrand, France, 21–24 October 2000; pp. 121–135.

Pascual-Alonso M, Miranda-de la Lama GC, Aguayo-Ulloa L, Villarroel M, Alierta S, Maria G. 2014. Influence of coat colour on Chamarita sheep phenotypes, behaviour, welfare and performances. Animal Genetic Resources. 54: 179–184.

Peralta-Lailson M, Trejo-González AA, Pedraza-Villagómez P, Berruecos-Villalobos JM, Vasquez CG. 2005. Factors affecting milk yield and lactation curve fitting in the creole sheep of Chiapas, Mexico. Small Ruminant Research. 58: 265–273.

Peterson SW, Kenyon PR, Morris ST, Lopez-Villalobos N, Morel PCH. 2005. Milk production in East Friesian-cross ewes lambing year round. Proceedings of the New Zealand Society of Animal Production. 65: 173–177. Available online: http://www.nzsap.org/proceedings/2005/milk-production-east-friesian-cross-ewes-lambing-year-round (accessed on 8 December 2022).

Pollott GE, Gootwine E. 2004. Reproductive performance and milk production of Assaf sheep in an intensive management system. Journal of Dairy Science. 87: 3690–3703.

Pulina G, Nudda A, Battacone G, Cannas A. 2006. Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk. Animal Feed Science and Technology. 131: 255–291.

Robles Jimenez LE, Angeles Hernandez JC, Palacios C, Abecia JA, Naranjo A, Osorio Avalos J, Gonzalez-Ronquillo M. 2020. Milk production of Lacaune sheep with different degrees of crossing with Manchega sheep in a commercial flock in Spain. Animals. 10: 520–530.

Rook AJ, Dhanoa MS, Gill M. 1990. Prediction of the voluntary intake of grass silages by beef cattle. Accuracy of alternative prediction models. Animal Science. 50: 455–466.

SAS. 2004. Statistical Analysis System, Version 9.4; SAS Institute Inc.: Cary, NC, USA.

Scatà MC, Catillo G, Annicchiarico G, De Matteis G, Napolitano F, Signorelli F, Moioli B. 2010. Investigation on lactation persistency and IGF-I gene polymorphisms in dairy sheep. Small Ruminant Research. 89: 7–11.

Schaeffer LR. 2004. Application of random regression models in animal breeding. Livestock Production Science. 86: 35–45.

Scholtens MR, Peterson S, Gunson K, Gunson A, Lopez-Villalobos N. 2018. Genetic evaluation of dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. 61: 81–91.

Silvestre AM, Petim-Batista F, Colaço J. 2006. The accuracy of seven mathematical functions in modeling dairy cattle lactation curves based on test-day records from varying sample schemes. Journal of Dairy Science. 89: 1813–1821.

Snowder GD, Glimp HA. 1991. Influence of breed, number of suckling lambs, and stage of lactation on ewe milk production and lamb growth under range of conditions. Journal of Animal Science. 69: 923–930.

Sölkner J, Fuchs W. 1987. A comparison of different measures of persistency with special respect to variation of test day milk yields. Livestock Production Science. 16: 305–319.

Swalve HH, Gengler N. 1999. Genetics of lactation persistency. Metabolic stress in dairy cows. Occasional Publication No. 24. British Society of Animal Science. 24: 75–82.

Van der Werf JHJ, Goddard ME, Meyer K. 1997. The use of covariance functions and random regression for genetic evaluation of milk production based on test day records. Journal of Dairy Science. 81: 3300–3308.

Velarde-Guillén J, López-Villalobos N, Sainz-Ramírez A, González-Sánchez M, Arriaga-Jordán CM, Albarrán-Portillo B. 2022. Factors affecting the lactation curve parameters of crossbred dairy ewes in the highlands of Mexico. Tropical Animal Health and Production. 54: 373.

Wolfinger R. 1993. Covariance structure selection in general mixed models. Communications in Statistics-Simulation and Computation. 22: 1079–1106.

Appendix 1 (Supplementary information for Chapter 3)

Appendix 1 Table 1. Least squares means (Mean) and standard errors (SE) of regression coefficients (a0, a1, 12, a3, a4) for lactation curve of milk, estimated for 130 days after weaning, for different ewe ages, litter size, coat colour, and days in TAD milking, at Kingsmeade farm, during the production season 2021-2022.

	Milk (kg)									
Effect	a0		a1		a2		a3		a4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age										
1	0.493[b]	0.041	-0.28[a]	0.04	0.163[bc]	0.035	-0.078	0.031	0.021	0.033
2	0.657[a]	0.027	-0.427[b]	0.026	0.241[a]	0.023	-0.1	0.02	0.055	0.021
3	0.706[a]	0.027	-0.447[b]	0.026	0.221[ac]	0.023	-0.06	0.02	0.035	0.022
>4	0.691[a]	0.022	-0.435[b]	0.022	0.217[ac]	0.019	-0.068	0.017	0.044	0.018
Litter size										
1	0.626	0.021	-0.382	0.021	0.198	0.018	-0.071	0.016	0.041	0.017
2	0.648	0.022	-0.412	0.021	0.223	0.018	-0.082	0.016	0.038	0.017
Coat colour										
Black	0.632	0.03	-0.411	0.029	0.24	0.025	-0.104	0.023	0.048	0.024
White	0.642	0.014	-0.383	0.014	0.181	0.0123	-0.05	0.011	0.03	0.011
[1]TAD days										
0	0.545[b]	0.024	-0.343[bc]	0.023	0.194	0.02	-0.075	0.018	0.012	0.019
14	0.665[a]	0.03	-0.409[ac]	0.029	0.198	0.025	-0.057	0.023	0.035	0.024
21	0.661[a]	0.03	-0.402[ac]	0.03	0.207	0.026	-0.074	0.023	0.051	0.024
28	0.726[a]	0.032	-0.465[a]	0.031	0.25	0.027	-0.098	0.024	0.072	0.026

[1]TAD days= days in twice-a-day milking. [a, b, c]Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Appendix 1 Table 2. Least squares means (Mean) and standard errors (SE) of regression coefficients (a0, a1, 12, a3, a4, a5) for lactation curve of fat (g), estimated for 130 days after weaning, for different ewe ages, litter size, coat colour, and days in TAD milking, at Kingsmeade farm, during the production season 2021-2022.

	Fat (g)											
Effect	a0		a1		a2		a3		a4		a5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age												
1	31.70[b]	2.31	-10.40[ac]	1.86	8.60	1.41	-2.49	1.27	0.55	1.31	-1.16[b]	1.42
2	38.40[a]	1.53	-13.80[bc]	1.23	10.00	0.93	-3.41	0.84	-1.21	0.86	3.79[a]	0.94
3	40.20[a]	1.53	-15.00[b]	1.23	9.18	0.93	-1.74	0.84	-1.30	0.87	2.55[ac]	0.94
>4	40.40[a]	1.28	-14.80[b]	1.03	8.97	0.78	-2.48	0.70	-0.10	0.72	1.73[ab]	0.78
Litter size												
1	37.70	1.20	-13.10	0.97	8.93	0.73	-2.49	0.66	-0.72	0.68	2.18	0.74
2	37.70	1.20	-13.90	1.00	9.49	0.75	-2.57	0.68	-0.30	0.70	1.26	0.76
Coat colour												
Black	36.96	1.70	-12.90	1.37	9.31	1.04	-3.07	0.94	-0.26	0.96	1.97	1.04
White	38.40	0.81	-14.10	0.65	9.11	0.49	-1.99	0.44	-0.77	0.46	1.48	0.49
[1]TAD days												
0	31.56[b]	1.36	-8.95b	1.09	7.88	0.83	-2.80	0.74	0.21[ac]	0.76	0.15[bc]	0.83
14	38.96[a]	1.71	-14.12a	1.37	8.87	1.04	-1.53	0.93	-2.04[bc]	0.95	2.88[a]	1.04
21	40.83[a]	1.72	-16.52a	1.38	10.35	1.05	-1.96	0.94	-1.38[ac]	0.96	2.52[ac]	1.05
28	42.68[a]	1.82	-16.84a	1.46	10.34	1.11	-3.60	0.99	0.62[a]	1.01	2.27[ac]	1.11

[1]TAD days= days in twice-a-day milking. [a, b, c]Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Appendix 1 Table 3. Least squares means (Mean) and standard errors (SE) of regression coefficients (a0, a1, 12, a3, a4, a5) for lactation curve of protein (g), estimated for 130 days after weaning, for different ewe ages, litter size, coat colour, and days in TAD milking, at Kingsmeade farm, during the production season 2021-2022.

	Protein (g)											
Effect	a0		a1		a2		a3		a4		a5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age												
1	27.67[b]	1.99	-11.76[a]	1.64	8.76	1.17	-2.75	1.03	1.84	1.14	-0.53	1.23
2	34.57[a]	1.32	-16.06[b]	1.08	9.90	0.77	-2.07	0.68	0.19	0.75	2.78	0.81
3	35.88[a]	1.32	-16.73[b]	1.08	9.02	0.77	-0.73	0.68	-0.10	0.76	2.17	0.81
>4	35.38[a]	1.10	-16.19[b]	0.91	9.05	0.65	-1.22	0.57	0.36	0.63	2.13	0.68
Litter size												
1	32.67	1.04	-14.32	0.85	8.79	0.61	-1.80	0.54	0.58	0.59	1.87	0.64
2	34.08	1.07	-16.06	0.88	9.57	0.63	-1.59	0.55	0.56	0.61	1.39	0.66
Coat colour												
Black	33.30	1.42	-15.80	1.21	10.45	0.86	-2.53	0.76	0.25	0.84	2.54	0.90
White	33.45	0.70	-14.57	0.57	7.91	0.41	-0.85	0.36	0.89	0.40	0.73	0.43
[1]TAD days												
0	29.15[c]	1.17	-12.98[b]	0.96	9.67[ac]	0.67	-2.64	0.60	0.51	0.67	1.24	0.72
14	34.36[b]	1.47	-15.09[b]	1.20	8.43[ac]	0.85	-0.88	0.76	0.08	0.84	2.20	0.90
21	34.10[b]	1.48	-14.98[b]	1.21	7.88[bc]	0.85	-0.82	0.76	0.99	0.85	0.86	0.91
28	38.19[a]	1.57	-18.85[a]	1.28	10.47[a]	0.90	-1.88	0.81	0.66	0.90	2.54	0.96

[1]TAD days= days in twice-a-day milking. [a, b, c]Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Appendix 1 Table 4. Least squares means (Mean) and standard errors (SE) of regression coefficients (a0, a1, 12, a3, a4) for lactation curve of lactose (g), estimated for 130 days after weaning, for different ewe ages, litter size, coat colour, and days in TAD milking, at Kingsmeade farm, during the production season 2021-2022.

	Lactose (g)									
Effect	a0		a1		a2		a3		a4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age										
1	23.49[b]	2.04	-14.65[a]	2.04	7.73	1.79	-4.19	1.56	1.27	1.71
2	31.98[a]	1.35	-22.69[b]	1.35	11.73	1.18	-5.21	1.03	3.13	1.13
3	34.05[a]	1.35	-23.49[b]	1.35	10.75	1.18	-3.17	1.03	1.98	1.13
>4	33.22[a]	1.13	-22.86[b]	1.13	10.47	0.99	-3.77	0.86	2.65	0.94
Litter										
1	30.14	1.06	-20.18	1.06	9.59	0.93	-3.79	0.81	2.30	0.89
2	31.23	1.09	-21.66	1.09	10.74	0.96	-4.38	0.84	2.21	0.91
Coat										
Black	30.51	1.50	-21.60	1.50	11.65	1.32	-5.40	1.15	2.59	1.26
White	30.85	0.71	-20.25	0.71	8.69	0.62	-2.78	0.55	1.92	0.60
[1]TAD										
0	26.34[b]	1.20	-18.57[a]	1.20	9.55	1.05	-4.14	0.19	0.88	1.01
14	32.22[a]	1.50	-21.48[ac]	1.50	9.24	1.32	-2.92	1.15	2.07	1.27
21	31.84[a]	1.52	-20.91[ac]	1.52	9.93	1.33	-3.90	1.16	2.86	1.28
28	34.70[a]	1.60	-24.04[bc]	1.60	12.18	1.40	-5.23	1.23	3.92	1.35

[1]TAD days= days in twice-a-day milking. [a, b, c]Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Appendix 1 Table 5. Number of ewes with 2, 3, and ≥ 4 test-day records during the 2021-2022 milk production season.

Total records	Number of ewes
2 records	9
3 records	29
≥ 4 records	131
Total:	169

Chapter 4

Animal factors affecting the cheese-making properties and the heat coagulation time of milk from dairy sheep in a New Zealand flock

This chapter has been published elsewhere. It has been reformatted and presented here with permission:

Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024. Animal factors affecting the cheese-making properties and the heat coagulation time of milk from dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. Published online: 27 Mar 2024. https://doi.org/10.1080/00288233.2024.2333826

Abstract

The objective of this study was to evaluate the effect of animal factors on the cheese-making properties and on the heat coagulation time of milk from individual dairy sheep in a New Zealand flock. A total of 521 individual records were obtained from a seasonal pasture-based flock of 169 ewes milked once-a-day, from 50 to 182 days in milk. A statistical model was used to quantify the effects of animal factors (coat colour variety, age, litter size, and stage of lactation) on the studied traits. Stage of lactation, confounded with seasonality, strongly influenced all properties of milk investigated. With the advancement of lactation, the milk took longer to coagulate after rennet addition, and the curd was softer. Higher relative cheese yield was achieved towards the end of lactation. The milk was also less stable to high temperature treatment in late lactation. Coefficient of variation for processability traits was high and ranged from 20.2 to 58%, which can be largely attributed to stage of lactation but could also indicate room for genetic improvement of traits. Further genetic studies are underway to define animal genetic variance, heritability, and the phenotypic and genetic correlations between these processability and milk composition traits.

Keywords

Milk coagulation; Cheese yield; Heat coagulation time; Milk composition; Dairy sheep; Stage of lactation; Seasonal.

4.1 Introduction

The New Zealand sheep dairy industry, initiated in the 1990s, is still in its early stages and can gain insights from global counterparts, and from the robust New Zealand cow dairy industry, which stands as one of the world's strongest (Lees and Lees 2018). Despite of few studies having investigated milk production of dairy sheep (Scholtens 2016; Marshall et al. 2023), there is a notable gap in research regarding the milk coagulation patterns and cheese-making efficiency of individual dairy sheep within New Zealand's pasture-based farming systems.

Several dairy sheep farms in New Zealand produce cheeses that are sold in the domestic market. Sheep milk is higher in total solids than cow milk and generates more cheese per litre (Park et al. 2007). The quantity and the quality of cheese produced will depend on

several factors, which include milk composition (percentages of fat, protein, casein, mineral content) and pH. These are known to vary with factors related to animal physiology (stage of lactation, age, litter size, health), animal genetics (breed, genetic variety, and genotype) and to the farming system (feed management, milking practices) (Bencini and Pulina 1997).

In cheese-making, chymosin hydrolyses the κ-casein proteins at the surface of the casein micelle, thereby removing the electrostatic stabilisation that keeps micelles as a colloidal suspension in milk. This leads to aggregation into flocs, precipitation, and formation of cheese curd. Cutting of the curd leads to the expulsion of liquid whey and firming of the protein gel. It is well known that the coagulation speed and strength of the gel formed are higher if the temperature is increased, pH is reduced, or Ca^{2+} concentration is increased (Lucey 2011). Milk coagulation properties (MCP) reflect the milk's suitability for cheese-making and are measured using a Formagraph (Foss Analytics). Additionally, micro-manufactured cheese yield from milk samples of individual animals can be measured to estimate the percentage of milk that is converted into a fresh soft curd (Othmane et al. 2002).

Although sheep milk is excellent for cheese-making, it is known to have lower stability under heat treatments when compared with cow milk. This is due to its high concentration of solids, and other factors such as casein micelle properties (size, mineralization, and composition), and denaturation of whey proteins (Raynal-Ljutovac et al. 2007). Stability of milk to ultra-high temperature treatment (135-140 °C, for 2-3 seconds) is relevant for processing into other dairy product such as infant formula and beverages. Heat stability refers to the ability of milk to withstand a heat treatment without coagulation, i.e. the formation of proteinaceous flocs that become visible particles in the milk (Huppertz 2016). Heating milk above 100 ºC causes several simultaneous reactions including dissociation of κ-casein from micelles, acidification, dephosphorylation of proteins and proteolysis, which collectively drive protein coagulation (Fox 1998). It is known that larger proportions of κ-casein, higher calcium, lower citrate and phosphate content, and lower proportions of urea reduce heat stability (Timlin et al. 2021).

Fluctuations in milk composition due to ewe physiology or due to seasonal changes can affect processability, especially in the context of grass-based seasonal milk production, where dietary patterns are influenced by weather conditions. Understanding variations in

coagulation behaviour among individual samples is valuable for identifying animals that produce milk with superior cheese-making ability. Therefore, the objective of this study was to evaluate the effects of animal factors (coat colour variety, age or parity, litter size, and stage of lactation) on milk rennet coagulation properties, relative cheese-yield, and heat coagulation time of milk from individual dairy sheep in a New Zealand flock.

4.2 Materials and methods

Data were collected from 169 ewes from a commercial flock in Masterton, Wairarapa, New Zealand. Ethics approval for this study was obtained (MUAEC Protocol 21/45). The farm operates on a pasture-based system with low supplementation. Pasture is composed of white clover and lucerne. During the production season, the metabolizable energy in the pasture decreased from 12.0 to 8.2 MJ/kg DM, crude protein declined from 26.7 to 14.1% DM throughout the milk production season, and NDF increased from 36.5 to 60.6% DM. The breed is mainly composed of East-Friesian genetics, and two varieties are observable: black or white ewes (Marshall et al. 2023). A minimum of 2 milk tests were obtained from each ewe from 50 to 182 days in milk. Test-day milk yield of individual animals was manually recorded, and a representative milk sample was taken. The milk samples were immediately refrigerated for transportation to Palmerston North. Sodium azide (at final concentration of 0.025%) was added to milk samples on arrival in Palmerston North and kept refrigerated. All analyses were performed within 3 days after milk collection.

4.2.1 Milk composition

An aliquot was analysed by Milk Test NZ Ltd (Hamilton, NZ) using a Combi FOSS instrument (Foss Analytics). The composition analysis included fat (%), protein (%), lactose (%), and somatic cell count (SCC, cells/mL). The analyses for casein (%), urea (mg/100mL), and citric acid (mg/100mL) were performed using a Fourier-transform Infrared (FTIR) milk-analyser MilkoScan FT6000 (Foss Analytics). Milk samples were also submitted at a contract laboratory (Massey Nutrition Lab) for analysis of total calcium content (mg/100 mL). Ratio of casein to calcium was calculated as casein (%) divided by total calcium content (mg/100 mL), multiplied by 100. Ratio of casein to protein was

calculated as casein (%) divided by protein (%), multiplied by 100, likewise for ratio of casein to fat. Somatic cell score (SCS) was calculated as SCS = Log_2(SCC).

4.2.2 Milk coagulation

Measures of traditional milk coagulation properties were obtained using a Formagraph (Foss Analytics). For each individual milk sample, 10 mL was heated to 31°C before the addition of the rennet solution at 0.0513 IMCU/mL of milk (Cipolat-Gotet et al. 2016). After rennet addition, analysis was continued for 30 minutes. The curd firmness (mm) of each sample was measured every 7.5 seconds. All measurements were performed in duplicates on the same day, and the average values were used for the analysis. The traditional milk coagulation parameters (MCPs) obtained from the Formagraph included rennet coagulation time (RCT, min), time to reach curd firmness of 20 mm (K20, min), and curd firmness at 30 minutes (A30, mm) (McMahon and Brown 1982).

Individual milk coagulation curves were fitted with the 2nd order plus dead time (SOPDT) model (Seborg et al. 2016) expressed as follows:

$$CF_t = CF_P\left(1 - \frac{\tau_1}{\tau_1 - \tau_2}e^{\left(\frac{-(t-RCT_{eq})}{\tau_1}\right)} + \frac{\tau_2}{\tau_1 - \tau_2}e^{\left(\frac{-(t-RCT_{eq})}{\tau_2}\right)}\right)$$

Where CFt is the curd firmness at time t (mm); CF_p is the asymptotical potential value of curd firmness at an infinite time (mm); τ_1 and τ_2 are the time constants; RCT_{eq} is the rennet coagulation time (dead time) equivalent to the RCT in traditional Formagraph (Sanjayaranj et al. 2023). The parameters of this equation were estimated using the Generalized Reduced Gradient algorithm implemented in the Solver module of Microsoft Excel for Microsoft 365 MSO (Version 2311 Build 16.0.17029.20140) setting up as the objective function to minimize the sum squares of errors (differences between predicted and actual values of the curd firmness curve).

4.2.3 Milk pH

Milk pH was measured at 31°C, using a pH meter (EcoScan Model pH5) on the same day as milk coagulation properties were obtained.

4.2.4 Individual laboratory cheese yield

A smaller set of a total of 376 samples, due to feasibility reasons, was processed for measurement of individual laboratory cheese yield (ILCY), as per Othmane et al. (2002). Raw milk was warmed to ambient temperature and 10 g weighed into test tubes (15 mm internal diameter) and then equilibrated at 31 °C for 10 min in a water bath. Rennet solution (40 µL) was added to the milk samples in the tubes, reaching a final dose of 0.060 IMCU/g. The tubes were closed and quickly inverted, to ensure uniform distribution of the rennet, and kept at 31 °C for 1 hour in a water bath. The formed coagulum was cut (inside the tube) and centrifuged at 4000 rpm for 15 min, to separate curd from whey. The whey was removed by draining with the test tube facing downwards. ILCY was expressed in % (w/w) of the relative weight of the centrifuge residue on the initial weighed milk.

4.2.5 Heat coagulation time

The heat coagulation time (HCT) of whole raw milk was defined as the time at which the sample coagulated after heating to 140 °C in an oil bath. An aliquot of 4 mL of milk was pipetted into a 10 mL screw-capped glass tube. The tube was then inserted into a rocking apparatus and submerged into the oil bath, as described by Cole and Tarassuk (1946).

4.2.6 Statistical analysis

Descriptive statistics (mean, standard deviation, minimum and maximum values, and coefficient of variation) for MCPs and composition traits were obtained in SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). Analyses of variances were performed using the MIXED procedure with a linear model that included the fixed effects of ewe coat colour as an indicator of variety within the breed (categorical variable with two levels: black or white), litter size (categorical variable with two levels: 1 lamb or 2 lambs and greater), ewe age (categorical variable with four levels: 1, 2, 3, and 4 years and older), and stage of lactation, which is a categorical variable with three levels: 1, 2, and 3, representing the different ranges of days in milk (date of test – date of lambing) from ≥ 50 and <95, ≥ 95 and <140, ≥ 140 and ≤ 182, respectively, and deviation from median lambing date as covariate. Random effects included effect of ewe and random residual error:

$$y_{ijklmn} = \mu + \text{coat colour}_i + \text{litter size}_j + \text{age}_k + \text{sol}_l + \text{dmld}_m + \text{ewe}_n + e_{ijklmn}$$

where y_{ijklmn} represents the dependent variable which include MCPs, ILCY, HCT, pH, composition traits (percentages of fat, protein, lactose, and casein), total calcium, urea, and SCS. The least squares means of traits were plotted across the different stages of lactation (mid- to late-lactation). The same linear model was used to obtain the least squares means and standard error of parameters obtained from the CF_t equation and used to obtain the curd firmness curves.

4.3 Results

The means, standard deviations, and the F-values and associated probabilities for each dependent variable from the analyses of variance are shown in Table 4.1. Significant ewe effect ($p < 0.01$) was observed for all dependent traits. Effect of stage of lactation was significant on all dependent traits ($p < 0.001$). Effects of age of ewe at lambing, coat colour, litter size and dmld were significant for some of the dependent traits.

The flock produced an average of 0.57 L of milk/day per ewe and 17.18% of total solids in milk. Coefficient of variation for gross milk composition traits ranged from 5.4 to 20.3%. Average SCS calculated as Log_2(SCC) was 16, and coefficient of variation was 12.6%. When calculated as 3 + Log_2(SCC/100) (Wiggans and Shook 1987) this average was 12.6. The actual average SCC was 390,000 cells/mL. In this flock, only 8% of the milk samples had SCC above 500,000 cells/mL.

Average RCT, K20, and A30 were 13.51 min, 2.75 min, and 52.64 mm, respectively. Only 5% of the samples did not coagulate within 30 minutes of rennet addition, and only 6.8% of the samples did not reach curd firmness of 20 mm. Non-coagulating samples and samples that did not reach 20 mm occurred, on average, at 132 days in milk, and had an average SCS of 18.5. Average cheese yield of the flock was 44.7%. Coefficient of variation for traits related to cheese-making ranged from 20.2 to 46.2%.

The individual milk samples from this flock took, on average, 1.43 minutes (1 minute and 26 seconds) to coagulate in an oil bath set at 140°C, at natural milk pH. There was large variation in this flock for this trait (58%).

Tables 4.2, 4.3, 4.4 and 4.5 present least squares means and standard errors of all dependent traits for the different animal factors considered in this study. Figure 4.1 depicts the least squares means of cheese-making properties, heat coagulation time, ILCY, gross milk composition, calcium content, milk urea, and citric acid at three stages of lactation. The patterns of milk curd firmness over time after rennet addition are presented in Figure 4.2, for different lactation stages, ewe coat colours, ewe ages, and litter sizes.

Table 4.1. Means, standard deviation (SD), coefficient of variation (CV) and F-value for effects of animal factors on milk yield and composition, SCS, pH, milk coagulation properties, cheese yield, and heat coagulation time for dairy sheep milked once-a-day in mid- and late-lactation during the 2021-2022 production season.

					F-value				
Trait[1]	N	Mean	SD	CV (%)	Coat colour	Age	Litter size	Stage of lactation[2]	dmld[3]
Milk yield (L/day)	521	0.57	0.25	43.9	0.83	8.5***	0.63	157.8***	5.1*
Fat (%)	521	6.30	1.27	20.3	1.0	0.4	3.9*	146.3***	5.3*
Protein (%)	521	5.52	0.68	12.3	1.1	4.9**	6.1*	269.4***	4.1*
Lactose (%)	521	4.72	0.27	5.7	0.0	0.6	0.1	326.8***	2.7
Total solids (%)	521	17.18	1.69	9.8	0.1	2.9*	0.0	190.0***	6.4**
Solids non-fat (%)	512	11.04	0.60	5.4	0.6	6.5***	6.0*	111.5***	1.6
Casein (%)	512	4.20	0.49	11.7	0.1	4.2**	5.3*	239.6***	1.0
Casein:Protein	521	76.5	2.1	2.7	27.8***	1.0	0.4	8.0***	6.0**
Casein:Fat	512	69.1	14.2	20.5	4.1*	1.2	15.8***	24.4***	0.1
Calcium (mg/100 mL)	521	185.7	25.3	13.6	1.0	4.5**	0.0	52.1***	1.7
Casein:Calcium	512	2.29	0.31	13.5	0.3	1.1	2.9	38.3***	4.0*
Citric acid(mg/100mL)	512	83.9	29.3	34	3.5	1.6	0.9	48.8***	0.5
Urea (mg/100mL)	512	39.82	7.58	19.0	2.9	0.1	0.0	53.6***	4.3*
SCS (Log_2SCC)	521	16.23	2.05	12.6	0.6	1.8	0.8	32.4***	10.6**
pH	521	6.59	0.09	1.4	4.2*	5.9***	0.4	155.3***	2.06
RCT (min)	494	13.51	3.41	25.2	10.6**	0.9	6.3**	20.4***	0.5
K20 (min)	487	2.75	1.27	46.2	0.24	2.1	4.5*	7.2***	4.7*
A30 (mm)	494	52.64	10.64	20.2	2.0	1.3	4.4*	11.9***	1.32
ILCY (%)	372	44.7	9.4	21.0	5.2*	7.9***	0.3	10.5***	0.6
HCT (min)	344	1.43	0.83	58.0	6.3**	1.2	1.3	19.4***	6.6**

[1] Casein:Protein= ratio of casein to protein. Casein:Fat= ratio of casein to fat. Casein:Calcium= ratio of casein to total calcium. SCS=somatic cell score; RCT= rennet coagulation time after rennet addition; K20= time to reach curd firmness of 20 mm after coagulation; A30= curd firmness at 30 min after rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time.

[2] Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).

[3] dmld: deviation from median lambing date of the flock (ewe lambing date – median lambing date of the flock).

Statistical significance is given as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4.2. Least squares means and standard errorss of milk coagulation properties, cheese yield, heat coagulation time and pH for different animal factors of dairy sheep milked once-a-day in mid- and late-lactation during the 2021-2022 production season.

Effect		Trait[1]					
		RCT (min)	K20 (min)	A30 (mm)	ILCY (%)	HCT (min)	pH
Coat colour	Black	15.18^{a} ± 0.50	2.84 ± 0.19	50.41 ± 1.52	47.37^{a} ± 1.25	1.79^{a} ± 0.12	6.61^{a} ± 0.01
	White	13.38^{b} ± 0.24	2.74 ± 0.09	52.76 ± 0.72	44.19^{b} ± 0.63	1.43^{b} ± 0.06	6.58^{b} ± 0.01
Age	1	14.95 ± 0.73	2.39 ± 0.27	53.11 ± 2.21	49.82^{a} ± 1.70	1.62 ± 0.16	6.63^{a} ± 0.01
	2	14.06 ± 0.44	2.69 ± 0.16	52.88 ± 1.33	47.89^{a} ± 1.17	1.69 ± 0.11	6.59^{a} ± 0.01
	3	14.34 ± 0.44	3.02 ± 0.17	50.03 ± 1.35	42.46^{b} ± 1.20	1.67 ± 0.11	6.60^{a} ± 0.01
	≥4	13.75 ± 0.37	3.04 ± 0.14	50.31 ± 1.14	42.93^{b} ± 0.98	1.46 ± 0.09	6.56^{b} ± 0.01
Litter size	1	13.72^{b} ± 0.37	2.61^{b} ± 0.14	52.98^{a} ± 1.12	46.09 ± 0.93	1.67 ± 0.09	6.59 ± 0.01
	2	14.83^{a} ± 0.35	2.96^{a} ± 0.13	50.19^{b} ± 1.05	45.46 ± 0.89	1.55 ± 0.08	6.60 ± 0.01
Stage of lactation[2]	1 (≥50 and <95)	13.26^{c} ± 0.27	2.57^{b} ± 0.12	53.79^{a} ± 0.97	43.44^{b} ± 1.29	2.00^{a} ± 0.13	6.65^{a} ± 0.01
	2 (≥95 and <140)	14.34^{b} ± 0.31	2.74^{b} ± 0.12	52.38^{a} ± 1.00	44.66^{b} ± 0.84	1.69^{b} ± 0.08	6.60^{b} ± 0.01
	3 (≥140 and ≤182)	15.23^{a} ± 0.32	3.06^{a} ± 0.14	48.58^{b} ± 1.14	49.22^{a} ± 0.98	1.14^{c} ± 0.09	6.53^{c} ± 0.01

[1] RCT= rennet coagulation time after rennet addition; K20= time to reach curd firmness of 20 mm; A30= curd firmness at 30 min after rennet addiction; ILCY= individual laboratory cheese yield; HCT= heat coagulation time.
[2] Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).
[a, b, c] Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Table 4.3. Least squares means and standard errors of milk yield, gross composition traits, and ratio of casein to fat, for different animal factors of dairy sheep milked once-a-day in mid- and late-lactation during the 2021-2022 production season.

Effect		Trait					
		MY[2] (L/day)	Fat (%)	Protein (%)	Lactose (%)	Casein (%)	Casein:Fat[3]
Coat colour	Black	0.50 ± 0.03	6.39 ± 0.12	5.68 ± 0.08	4.67 ± 0.03	4.25 ± 0.06	70.5^{a} ± 1.6
	White	0.53 ± 0.01	6.52 ± 0.06	5.58 ± 0.04	4.68 ± 0.01	4.27 ± 0.03	66.9^{b} ± 0.8
Age	1	0.33^{b} ± 0.04	6.54 ± 0.18	5.81^{a} ± 0.12	4.68 ± 0.04	4.39^{a} ± 0.09	69.2 ± 2.4
	2	0.53^{a} ± 0.02	6.50 ± 0.11	5.76^{a} ± 0.07	4.70 ± 0.02	4.34^{a} ± 0.05	70.6 ± 1.4
	3	0.59^{a} ± 0.02	6.35 ± 0.11	5.46^{b} ± 0.07	4.66 ± 0.03	4.16^{b} ± 0.05	67.4 ± 1.4
	≥4	0.59^{a} ± 0.02	6.43 ± 0.09	5.48^{b} ± 0.06	4.66 ± 0.02	4.15^{b} ± 0.04	67.6 ± 1.2
Litter size	1	0.52 ± 0.02	6.57^{a} ± 0.09	5.53^{b} ± 0.06	4.67 ± 0.02	4.20^{b} ± 0.04	65.9^{b} ± 1.2
	2	0.50 ± 0.02	6.34^{b} ± 0.08	5.72^{a} ± 0.06	4.68 ± 0.02	4.32^{a} ± 0.04	71.6^{a} ± 1.1
Stage of lactation[1]	1 (≥50 and <95)	0.68^{a} ± 0.02	5.52^{c} ± 0.09	5.14^{c} ± 0.05	4.88^{a} ± 0.02	3.92^{c} ± 0.03	74.2^{a} ± 1.2
	2 (≥95 and <140)	0.50^{b} ± 0.02	6.30^{b} ± 0.09	5.52^{b} ± 0.05	4.74^{b} ± 0.02	4.18^{b} ± 0.03	68.2^{b} ± 1.2
	3 (≥140 and ≤182)	0.36^{c} ± 0.02	7.55^{a} ± 0.10	6.22^{a} ± 0.05	4.41^{c} ± 0.02	4.69^{a} ± 0.04	63.8^{c} ± 1.4

[1] Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).
[2] MY= daily milk yield.
[3] Casein:Fat= ratio of casein to fat.
[a, b, c] Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Table 4.4. Least squares means and standard errors of ratio of casein to protein, ratio of casein to calcium, contents of calcium, citric acid, and urea, and somatic cell score, for different animal factors of dairy sheep milked once-a-day in mid- and late-lactation during the 2021-2022 production season.

Effect		Trait					
		Casein:Protein[2]	Casein:Calcium[3]	Calcium (mg/100mL)	Citric acid (mg/100mL)	Urea (mg/100mL)	SCS[4] (Log_2SCC)
Coat colour	Black	75.2^{b} ± 0.3	2.30 ± 0.04	185.9 ± 3.3	75.9 ± 4.6	40.71 ± 0.96	16.5 ± 0.2
	White	76.8^{a} ± 0.1	2.28 ± 0.02	189.5 ± 1.6	85.2 ± 2.2	38.93 ± 0.46	16.3 ± 0.1
Age	1	74.1 ± 1.6	2.23 ± 0.06	197.7^{a} ± 4.9	87.1 ± 6.8	40.11 ± 1.42	16.7 ± 0.3
	2	74.1 ± 1.0	2.30 ± 0.04	189.8^{ab} ± 2.9	75.5 ± 4.1	39.80 ± 0.85	16.1 ± 0.2
	3	75.9 ± 1.0	2.30 ± 0.04	182.9^{bc} ± 3.0	76.3 ± 4.1	39.89 ± 0.85	16.2 ± 0.2
	≥4	74 .9 ± 0.8	2.34 ± 0.03	180.4^{c} ± 2.5	83.6 ± 3.4	39.48 ± 0.71	16.6 ± 0.2
Litter size	1	74.8 ± 0.8	2.26 ± 0.03	187.7 ± 2.4	82.6 ± 3.4	39.87 ± 0.71	16.3 ± 0.2
	2	74.7 ± 0.7	2.32 ± 0.03	187.7 ± 2.3	78.6 ± 3.2	39.77 ± 0.67	16.5 ± 0.2
Stage of lactation[1]	1 (≥50 and <95)	76.3^{a} ± 0.2	2.15^{b} ± 0.03	182.5^{b} ± 2.1	92.1^{a} ± 2.8	43.55^{a} ± 0.64	15.6^{c} ± 0.1
	2 (≥95 and <140)	76.0^{b} ± 0.2	2.35^{a} ± 0.03	179.0^{b} ± 2.2	78.0^{b} ± 2.8	39.98^{b} ± 0.66	16.6^{b} ± 0.1
	3 (≥140 and ≤182)	75.6^{c} ± 0.2	2.37^{a} ± 0.03	201.6^{a} ± 2.4	71.7^{c} ± 3.0	35.93^{c} ± 0.74	17.1^{a} ± 0.2

[1] Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).
[2] Casein:Protein= ratio of casein to protein.
[3] Casein:Calcium= ratio of casein to total calcium.
[4] SCS=somatic cell score.
[a, b, c] Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Table 4.5. Least squares means and standard errors of parameters describing the curves of curd firmness for ewe coat colour varieties, ages, litter sizes, and stages of lactation of dairy sheep milked once-a-day in mid- and late-lactation during the 2021-2022 production season.

Effect		CF_t parameter[2]			
		CF_P	τ_1	τ_2	RCT_{eq}
Coat colour	Black	51.75 ± 1.48[b]	0.29 ± 0.03	6.68 ± 0.34	14.80 ± 0.39[a]
	White	54.99 ± 0.75[a]	0.25 ± 0.01	6.49 ± 0.17	13.36 ± 0.20[b]
Age	1	52.80 ± 1.87[b]	0.27± 0.03	6.43 ± 0.43	14.60 ± 0.49
	2	57.19 ± 1.36[a]	0.29 ± 0.02	6.28 ± 0.31	13.91 ± 0.36
	3	52.71 ± 1.33[b]	0.26 ± 0.02	6.67 ± 0.31	14.15 ± 0.35
	≥4	50.78 ± 1.18[b]	0.28 ± 0.02	6.98 ± 0.27	13.64 ± 0.31
Litter size	1	53.98 ± 1.12	0.29 ± 0.02	6.27 ± 0.26[b]	13.49 ± 0.29[b]
	2	52.76 ± 1.06	0.26 ± 0.02	6.90 ± 0.24[a]	14.67 ± 0.28[a]
Stage of lactation[1]	1 (≥50 and <95)	55.65 ± 1.08[a]	0.32 ± 0.02[a]	5.96 ± 0.25[b]	12.80 ± 0.28[b]
	2 (≥95 and <140)	54.66 ± 1.18[a]	0.22 ± 0.02[bc]	6.67 ± 0.27[a]	14.31 ± 0.31[a]
	3 (≥140 and ≤182)	49.81 ± 1.44[b]	0.28 ± 0.03[ac]	7.13 ± 0.33[a]	15.13 ± 0.38[a]

[1] Stage of lactation: categorical variable representing three different ranges of days in milk (date of test – date of lambing).
[2] CF_P: asymptote or maximum potential curd firmness at infinite time. τ_1 and τ_2: time constants. RCT_{eq}: coagulation time (dead time) equivalent to the RCT in traditional Formagraph.
[a, b, c] Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

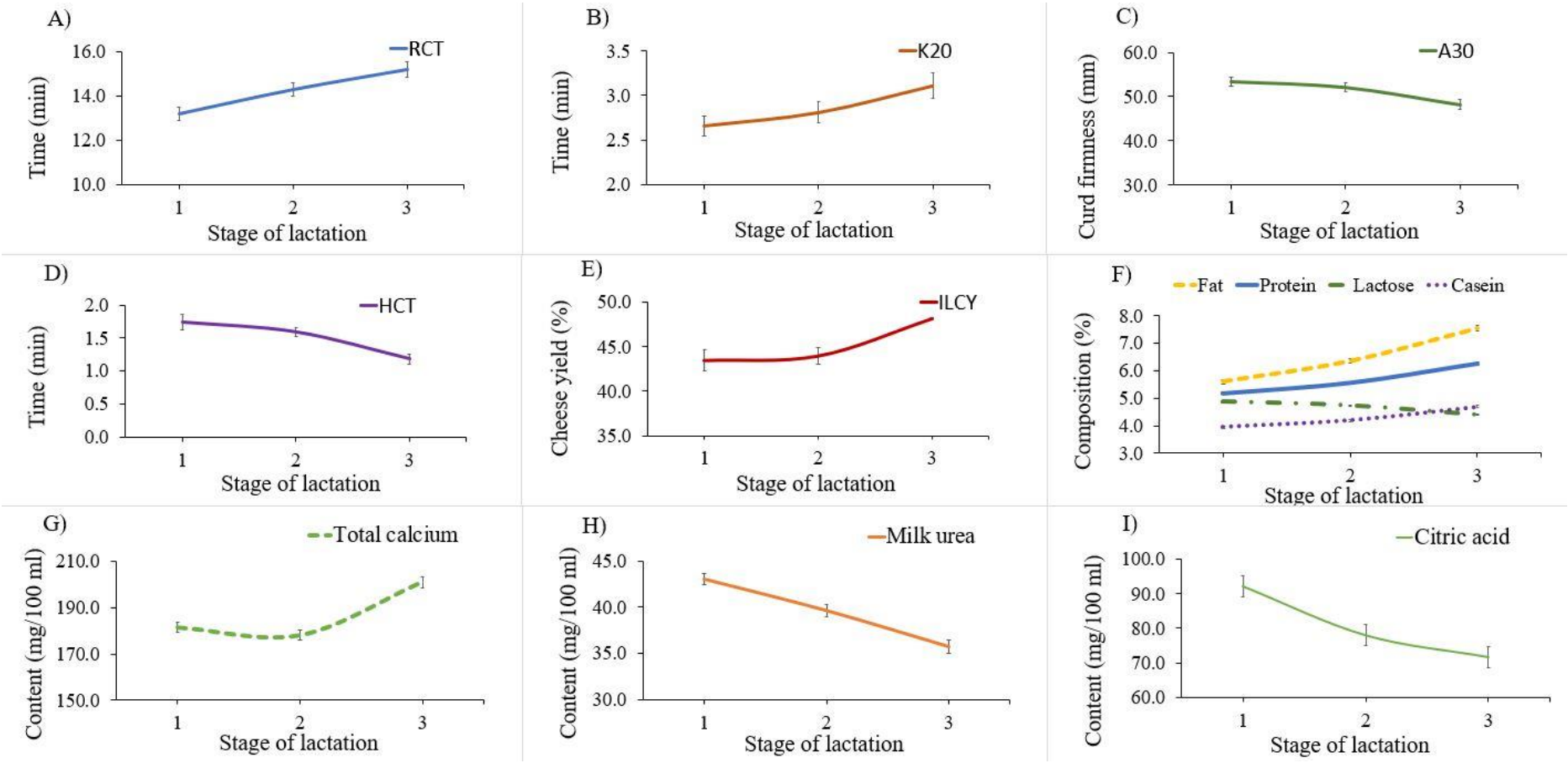


Figure 4.1. Least squares means of milk coagulation properties (RCT, K20, A30), heat coagulation time (HCT), individual laboratory cheese yield (ILCY), gross milk composition (contents of fat, protein, lactose, and casein), total calcium content, milk urea and citric acid contents, at three stages of lactation (1= ≥50 and <95 days in milk; 2= ≥95 and <140 days in milk; 3= ≥140 and ≤182 days in milk).

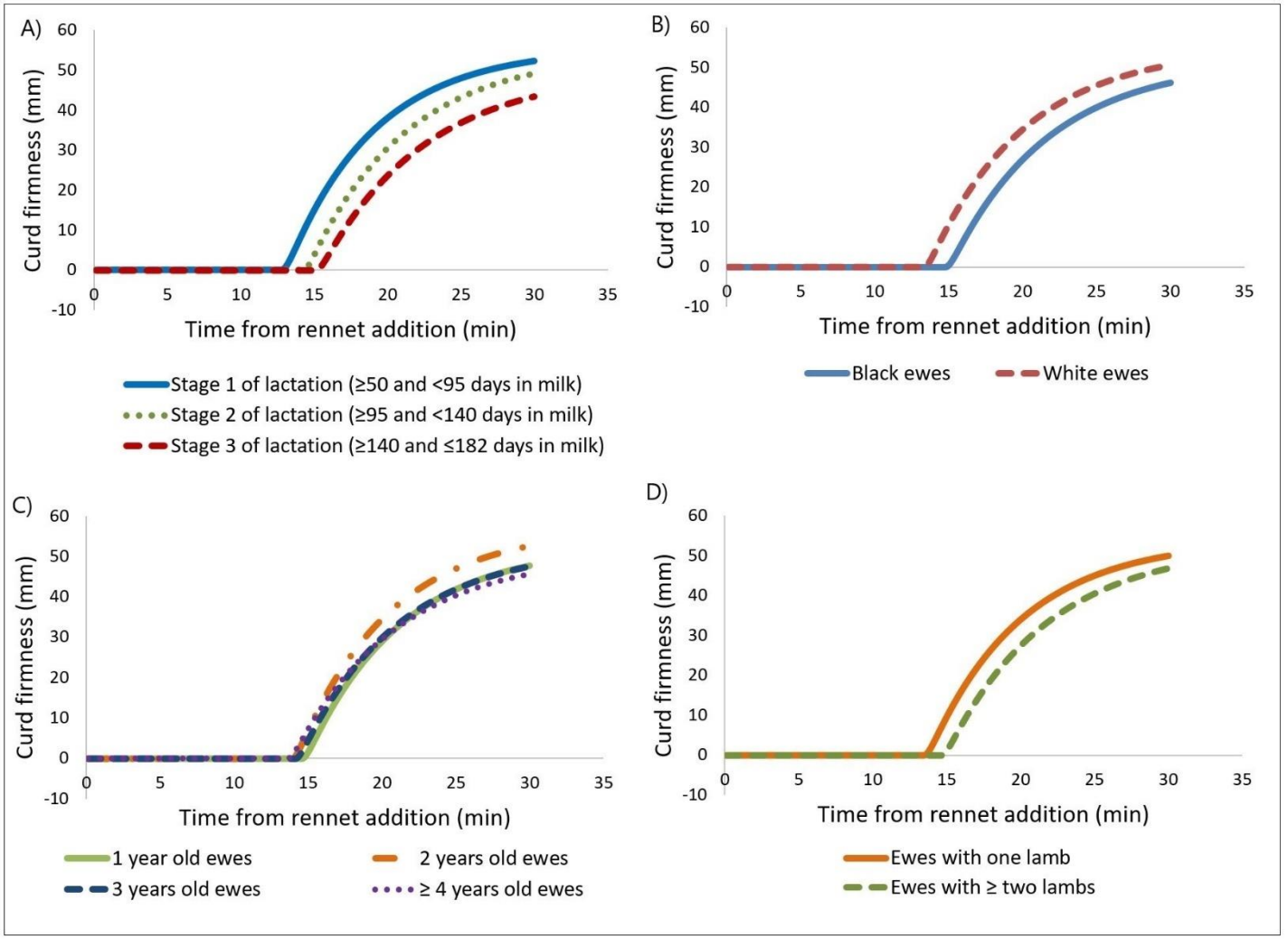


Figure 4.2. The patterns of milk curd firmness over time after rennet addition, for different lactation stages (A), ewe coat colours (B), ewe ages (C), and litter sizes (D).

4.4 Discussion

4.4.1 Flock performance for composition traits

The level of milk production per ewe in the season was previously discussed (Marshall et al. 2023, Chapter 3). Average gross milk composition was within the expected range for dairy sheep (Scholtens 2016; McCoard et al. 2023). Amongst the traditional milk components, fat was the most variable. Fat percentage is largely affected by changes in NDF content of the diet (Nudda et al. 2020).

Average SCS was within the wide physiological range reported for other populations of sheep. However, there is still no agreement on the acceptance threshold of SCC for sheep milk destined for cheesemaking, and there is still no regulation in New Zealand for bulk sheep milk. Moderate variation of SCS was found. Physiological variation in SCC occurs, being high at the beginning and at the end of lactation (Kaskous et al. 2023).

Average calcium content was similar with the literature for sheep milk, which is about 60-70% higher than average calcium content of cow milk (Park et al. 2007; McCoard et al. 2023). Moderate variation was observed for calcium content.

Average MU was also within the physiological range for dairy sheep (Cannas 2004). However, MU had large variation, with minimum and maximum values surpassing thresholds linked to impaired reprodution (>40–50 mg/dl) and to insufficient dietary protein and low milk production (<25–30 mg/dl) (Cannas 2004). Milk urea is an indicator of nitrogen intake and utilization, and a negative correlation is reported with milk protein. Milk urea is largely influenced by the balance between level of crude protein and energy content of the diet (Nudda et al. 2020).

4.4.2 Flock performance for processability traits

Similar to the findings of the present study, Manca et al. (2016), have reported a low percentage of non-coagulating milk samples among dairy sheep. However, it is worth noting that another study reported a higher percentage, reaching 19.42% (Garzón et al. 2021). Average RCT was similar to 13.39 minutes reported for Sarda ewes (Manca et al. 2016). Longer RCT values have also been reported, ranging from 17-31 minutes (Pelayo et al. 2021; Jiménez et al. 2023). Average K20 was longer than the range of 0.40-1.75 min (Manca et al. 2016; Pelayo et al. 2021), but shorter than the range of 4.47-8.11 min

observed by others (Sánchez-Mayor et al. 2019; Jiménez et al. 2023). Lower values for A30 of 12-28 mm have been reported (Sánchez-Mayor et al. 2019; Jiménez et al. 2023). It is important to note that differences in some of these reported results are not only attributed to different breeds and farming systems, but also to final dosages of IMCU and different temperature used in the analyses of MCPs in these studies. This flock of dairy sheep performed, on average, better than dairy cows for MCPs (Bittante et al. 2015; Sanjayaranj et al. 2023).

Relative cheese yield (ILCY) obtained from this flock was high compared to other reports (Vacca et al. 2019; Pelayo et al. 2021). Methods like the one used in the present study, however, tend to overestimate industrial cheese yield due to increased moisture retention, consequence of the lower temperature and small volume of milk used, and increased fat retention due to centrifugation step (Cipolat-Gotet et al. 2016). Average milk pH was 6.58, which is within the pH range of 6.51 – 6.85 for sheep milk (Park et al. 2007).

There are no other reports on HCT of individual raw sheep milk samples for comparison, only for bulk skim sheep milk (Pan et al. 2023), later discussed.

4.4.3 Effect of stage of lactation

Stage of lactation, naturally confounded with seasonality in grazing systems, had a strong significant effect and explained most of the variation for milk composition, consequently impacting MCPs and HCT. Average MY decreased by nearly half from mid- to late-lactation. This reduction in MY was previously discussed (Marshall et al. 2023, Chapter 3). Whereas FP and PP increased, due to the concentration effect. Fat percentage increased in late season also probably due to the increase in fibre content of pasture throughout the season. In opposite direction, lactose percentage decreased in late season. Lactose is an osmotic regulator and its secretion usually remains constant, however, decrease in late lactation has also been reported (Auldist et al. 1996) and this is suggested to be due to a parallel increase of milk salts diffusion when the mammary epithelial cells become damaged (Timlin et al. 2021).

The RCT and K20 increased by 2 and 0.5 minutes, respectively, and A30 decreased by 5 mm, in agreement with previous findings for ewe milk (Manca et al. 2016, Sevi et al. 2004). On the other hand, ILCY increased by 3.2% in late lactation, indicating a higher

moisture content in the curd due to the deterioration of fresh curd quality. Heat coagulation time significantly reduced, in the latest stage of lactation (stage 3), the milk coagulated 52 seconds faster, consistent with findings observed in cows (Loveday et al. 2021).

The casein content is crucial for cheese production, with a significant emphasis on its ratios with other components like calcium, total protein, and fat, which may have greatly impacted MCPs. Although protein and calcium contents increased in late lactation, the higher ratio of casein to total calcium in late lactation suggests a greater proportion of available calcium being bound to the micelle rather than in the soluble phase, which could have significantly impaired rennet coagulation (Lucey 2011). Although seasonal variations in pasture calcium content have been observed (Aston et al. 1931), ewes are able to compensate for nutritional deficits in calcium due to bone demineralization. The increasing concentration of major soluble minerals in milk in late lactation is suggested to be a result from the decreasing effectiveness of tight junctions between mammary epithelial cells as lactation progresses (Hettinga 2019).

Additionally, the reduction in the ratio of casein to protein indicates an elevation in whey protein content which may have impaired MCPs in late lactation. This increase in whey concentration in late lactation is also associated with heightened permeability of the mammary epithelium (Auldist et al. 1996). Whereas the impact of the ratio of casein to fat on rennet coagulation properties is contradictory, and is likely to be dependent on the size of casein micelles and of milk fat globules (Logan et al. 2015), these are determined by the protein and lipid compositions, respectively, which were not evaluated in the present study.

The decrease in milk pH from mid-to late-lactation agrees with another study (Albenzio et al. 2009), but different trends were also noted (Kuchtík et al. 2008). The decrease in milk pH may elucidate the decrease in heat stability, a phenomenon highly dependent on pH levels (Huppertz 2016). The pH of milk reflects the quantity of protons present, and the partitioning of minerals in the soluble phase should be investigated for a better understanding of natural variation in milk pH. Studies on bulk skim sheep milk samples showed maximum HCT of sheep skim milk at pH of 6.9, with heat stability reducing at higher or lower pH values (Pan et al. 2023). Measuring titratable acidity could also provide information on the buffering capacity of milk and the hygienic quality of it.

The reduction in MU with the progress of lactation may have impacted both HCT and MCPs. Milk urea can have a beneficial impact on milk heat stability because of its buffering effect (Huppertz 2016). The decrease in citric acid might also have contributed to lower HCT in late lactation, but to a lesser extent than MU (Fox 1998). Regarding the impact of MU on MCPs, previous studies have associated it with firmer A30 (Bland et al. 2015) and shorter RCT (Poulsen et al. 2015) in cow`s milk. It is possible that certain concentration of MU can reduce the second phase of rennet coagulation, but the opposite is also true in cases of very high MU (McGann and Fox 1974). The reduction in MU with the progress of lactation is likely to be due to the decrease in the content of crude protein (CP) in the pasture dry matter from Spring (early lactation) until late Summer (Marshall et al. 2023), and a more favourable balance between CP and energy content of the ewes' diet.

The worsening of MCPs and HCT might have been magnified by the physiological increase in SCC at the end of lactation. The presence of SCC in milk per se impairs whey drainage from the curd, resulting in retained moisture content (Albenzio et al. 2004). Also, in cases of drastic MY reduction, the mammary epithelium may be compromised during mammary involution in late lactation, causing an influx of blood components into the milk in a similar way to mastitis (Auldist et al. 1996). Differential cell count and microbiological analyses offer additional insights into milk quality for processing and help distinguish milk with high SCC due to mastitis.

4.4.4 Effect of age

Age (or parity) did not show any significant effect on any of MCPs but did impact CF_P, which is consistent with findings reported for cows (Bittante et al. 2015). CF_P was notably higher in the second parity, decreasing thereafter, possibly due to the lower calcium concentration in the milk of older ewes. Other studies have reported a significant effect of parity on MCPs, showing a deterioration of MCPs with the aging of ewes (Jaramillo et al., 2008; Pazzola et al., 2014), which aligns with the findings of the present study.

Mature ewes yielded more milk than one year-old ewes (Marshall et al. 2023), and produced milk with lower PP, while FP remained unaffected by age, in line with findings by Bittante et al. (2015). Additionally, mature ewes exhibited lower calcium levels in their

milk. The lower milk production of first parity ewes may be due to the still developing mammary glands and nutrient allocation for body growth rather than milk production.

The significant lower PP in milk of older ewes could be due to the dilution effect. The lower calcium concentration in the milk of older ewes could be due to the limited capacity to absorb calcium in the intestine and to mobilize calcium from bone reserves (Braithwaite and Riazuddin 1971). Consequently, older ewes (three- and four-years-old) also produced significantly less ILCY than the younger ewes (one- and two-years-old).

4.4.5 Effect of litter size

Ewes with twin lambs typically yield more milk, resulting in less concentrated milk compared to those with single lambs, due to increased secretion of placental lactogen and mammary gland development of twin bearing ewes (Abecia and Palacios 2017). In this study, however, litter size did not impact MY, probably because these differences are pronounced in earlier stages of lactation.

Despite this, litter size significantly affected major milk composition (fat, protein, and casein percentage). The milk from twin-bearing ewes had 0.19% more protein and 0.23% less fat, compared to milk from single-bearing ewes, in agreement with Fuertes et al. (1998). For Bittante et al. (2015), twin bearing ewes also produced milk with greater protein and non-fat solids contents. There was no difference in MU between single- and twin-bearing ewes and therefore no difference in energy balance status.

Litter size also significantly affected all MCPs and RCT_{eq}. The milk from single bearing ewes coagulated 1 min faster, and had slightly firmer curd, compared to milk from twin bearing ewes. This agrees with other studies that found low protein to be correlated to short coagulation time (Vacca et al. 2019), which at constant total calcium content can be explained by the partitioning of more calcium in the serum, which strengthens ionic shielding and salt-bridging effects that accelerate coagulation. Bittante et al. (2015) noted no differences in CF_P in milk samples from twin or single bearing ewes, but there was more rapid syneresis of milk from twin bearing ewes. In the present study, syneresis was not observed during the Formagraph analyses.

4.4.6 Effect of coat colour variety

Artificial selection based on adaptation to an extensive production system, rather than for pure white wool, allows for the retention of variations in coat colour in a flock, which some farmers link to production traits. A few studies with quantitative analyses have been made to support this (Pascual-Alonso et al. 2014). Furthermore, different levels of tolerance to heat-stress between white and black dairy animals can result in different levels of milk production (Arenas-Báez et al. 2023). Bernabucci et al. (2015) demonstrated how heat stress (and compositional changes to pasture in hot temperatures) affected coagulating parameters, with reduction in casein concentration, and changes in protein fractions.

In the present study, coat colour did not significantly affect milk production or major milk composition, except for the ratio of casein to protein, and casein to fat. On the other hand, coat colour was a significant effect on processability traits, including RCT, HCT ($p < 0.01$), ILCY, pH, CF_P and RCT_{eq} ($p < 0.05$).

Milk from white ewes had a greater proportion of casein in protein, lower ratio of casein to fat, coagulated 1.8 minutes earlier by rennet (RCT) and produced curd that was 3.24 mm firmer (CFP). Milk from white sheep, however, resulted in less ILCY, pointing out again to an unfavourable correlation between fresh curd quality and quantity. This is especially true for the method used for estimation of cheese yield, that tends to retain high moisture content. Also, milk from white ewes coagulated 0.36 minutes (21 seconds) earlier by heat.

4.5 Conclusion

Several ewe physiological and environmental factors are known to affect milk production and composition, consequently affecting the processability of milk, and should not be overlooked by the dairy sheep industry. In this study, stage of lactation, naturally confounded with seasonality, strongly impacted the properties of milk for cheese making and milk heat stability. Key mechanisms that may be influencing the variation observed in processability of milk includes variations in milk pH (which is highly determined by minerals in milk soluble phase), and factors contributing to the buffering capacity of milk. The ratios of casein to calcium and to total protein are of notable importance. Somatic cell count can impair milk processability directly or indirectly. Furthermore, protein

composition, lipid and mineral profiles should be investigated. Further studies are underway to investigate milk protein profile, and to define animal genetic variance, heritability, and genetic and phenotypic correlations between the production, composition, and technological traits for this flock of dairy sheep.

References

Abdelrahman MM 2008. The effect of high calcium intake by pregnant Awassi ewes at late gestation on minerals status and performance of ewes and newborn lambs. Livestock Science. 117: 15-23. doi: 10.1016/j.livsci.2007.11.006

Abecia J, Palacios C. 2017. Ewes giving birth to female lambs produce more milk than ewes giving birth to male lambs. Italian Journal of Animal Science. 17: 736-739. doi: 10.1080/1828051X.2017.1415705

Albenzio M, Caroprese M, Santillo A, Marino R, Taibi L, Sevi A 2004. Effects of somatic cell count and stage of lactation on the plasmin activity and cheese-making properties of ewe milk. Journal of Dairy Science. 87: 533-542.

Albenzio M, Santillo A, Caroprese M, d'Angelo F, Marino R, Sevi A 2009. Role of endogenous enzymes in proteolysis of sheep milk. Journal of Dairy Science. 92: 79-86. doi:10.3168/jds.2008-1439

Arenas-Báez P, Torres-Hernández G, Castillo-Hernández G, Hernández-Rodríguez M, Sánchez-Gutiérrez RA, Vargas-López S, González-Maldonado J, Domínguez-Martínez PA, Granados-Rivera LD, Maldonado-Jáquez JA 2023. Coat color in local goats: influence on environmental adaptation and productivity, and use as a selection criterion. Biology 12 pages. doi: 10.3390/biology12070929

Auldist MJ, Coats S, Sutherland BJ, Mayes JJ, McDowell GH, Rogers GL 1996. Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of Cheddar cheese. Journal of Dairy Research. 63: 269-280. doi: 10.1017/S0022029900031769

Bencini R and Pulina G 1997. The quality of sheep milk: A review. Australian Journal of Experimental Agriculture. 37: 485-504.

Bernabucci U, Basiricò L, Morera P, Dipasquale D, Vitali A, Cappelli FP, Calamari L 2015. Effect of summer season on milk protein fractions in Holstein cows. Journal of Dairy Science. 98: 1815–1827.

Bittante G, Cipolat-Gotet C, Malchiodi F, Sturaro E, Tagliapietra F, Schiavon S, Cecchinato A 2015. Effect of dairy farming system, herd, season, parity, and days in milk on modeling of the coagulation, curd firming, and syneresis of bovine milk. Journal of Dairy Science. 98: 2759-2774. doi: 10.3168/jds.2014-8909

Bland JH, Grandison AS, Fagan CC 2015. Evaluation of milk compositional variables on coagulation properties using partial least squares. Journal of Dairy Research. 82: 8-14. doi: 10.1017/S0022029914000508

Braithwaite G, Riazuddin S 1971. The effect of age and level of dietary calcium intake on calcium metabolism in sheep. British Journal of Nutrition. 26: 215-225. doi: 10.1079/BJN19710028

Cannas A 2004. Dairy Sheep Nutrition: Feeding of lactating ewes. pp. 79-108. Wallingford UK: CABI Publishing.

Cipolat-Gotet C, Cecchinato A, Stocco G, Bittante G 2016. The 9-MilCA method as a rapid, partly automated protocol for simultaneously recording milk coagulation, curd firming, syneresis, cheese yield, and curd nutrients recovery or whey loss. Journal of Dairy Science. 99: 1065-1082. doi: 10.3168/jds.2015-9734

Cole WC and Tarassuk NP 1946. Heat coagulation of milk. Journal of Dairy Science 29: 421-429. doi: 10.3168/jds.S0022-0302(46)92498-8

Fox PF, Mcsweeney PL, Paul L. 1998. Dairy chemistry and biochemistry. Springer: London, UK.

Fuertes J, Gonzalo C, Carriedo J, San Primitivo F 1998. Parameters of test day milk yield and milk components for dairy ewes. Journal of Dairy Science. 81: 1300-1307. doi: 10.3168/jds.S0022-0302(98)75692-9

Garzón A, Figueroa A, Caballero-Villalobos J, Angón A, Arias R, Perea JM 2021. Multivariate indices of milk properties in Manchega dairy sheep milk. Journal of Dairy Science. 104: 8618-8629. doi: 10.3168/jds.2021-20303

Hettinga KA 2019. Lactose: Lactose in the dairy production chain. Elsevier, Amsterdam, p 231-266.

Huppertz T 2016. Heat stability of milk. Advanced Dairy Chemistry: volume 1B: Proteins: Applied Aspects: 179-196.

Jaramillo D, Zamora A, Guamis B, Rodríguez M, Trujillo A 2008. Cheesemaking aptitude of two Spanish dairy ewe breeds: Changes during lactation and relationship between physico-chemical and technological properties. Small Ruminant Research 78: 48-55. doi: 10.1016/j.smallrumres.2008.04.005

Jiménez L, Caballero-Villalobos J, Garzón A, Oliete B, Pérez-Guzmán MD, Arias R 2023. Exploring the relationships between coagulation, composition, and hygienic quality of bulk tank milk from Manchega sheep. Small Ruminant Research. Volume: 228. doi: 10.1016/j.smallrumres.2023.107106

Kaskous S, Farschthchi S, Pfaffl MW 2023. Physiological aspects of milk somatic cell count in small ruminants- a review. Dairy. 4: 26-42. doi: 10.3390/dairy4010002

Ketto IA, Abdelghani A, Johansen AG, Skeie SB, Øyaas J 2019. Effect of milk protein genetic polymorphisms on rennet and acid coagulation properties after standardisation of protein content. International Dairy Journal. 88: 18-24.

Kuchtík J, Sustova K, Urban T, Zapletal D. 2008. Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. Czech Journal of Animal Science. 53: 55.

Lees N, Lees I 2018. Competitive advantage through responsible innovation in the New Zealand sheep dairy industry. International Food and Agribusiness Management Review 21: 505-524. doi: 10.22434/IFAMR2017.0013

Logan A, Leis A, Day L, Øiseth SK, Puvanenthiran A, Augustin MA. 2015. Rennet gelation properties of milk: Influence of natural variation in milk fat globule size and casein micelle size. International Dairy Journal. 46: 71-77.

Lucey J. 2011. Cheese: rennet-induced coagulation of milk. Elsevier, Madison, WI, USA. P 579-584.

Manca M, Serdino J, Gaspa G, Urgeghe P, Ibba I, Contu M, Fresi P, Macciotta N 2016. Derivation of multivariate indices of milk composition, coagulation properties, and individual cheese yield in dairy sheep. Journal of Dairy Science. 99: 4547-4557. doi: 10.3168/jds.2015-10589

Marshall AC, Lopez-Villalobos N, Loveday SM, Ellis A, McNabb W 2023. Modelling Lactation Curves for Dairy Sheep in a New Zealand Flock. Animals. 13: 349. doi: 10.3390/ani13030349

McCoard S, Stevens D, Selbie D, Day L, Young W, Bekhit AE-D, Samuelsson L 2023. Supporting the growth of the dairy sheep industry in New Zealand–industry update and review of a programme linking industry and science. New Zealand Journal of Agricultural Research: 1-35. doi: 10.1080/00288233.2023.2272594

McGann T, Fox P 1974. Physico-chemical properties of casein micelles reformed from urea-treated milk. Journal of Dairy Research. 41: 45-53. doi:10.1017/s0022029900014904

McMahon DJ, Brown RJ 1982. Evaluation of Formagraph for comparing rennet solutions. Journal of Dairy Science. 65: 1639-1642. doi: 10.3168/jds.S0022-0302(82)82390-4

Noce A, Pazzola M, Dettori ML, Amills M, Castelló A, Cecchinato A, Bittante G, Vacca GM. 2016. Variations at regulatory regions of the milk protein genes are associated with milk traits and coagulation properties in the Sarda sheep. Animal Genetics. 47: 717-726.

Nudda A, Atzori AS, Correddu F, Battacone G, Lunesu MF, Cannas A, Pulina G 2020. Effects of nutrition on main components of sheep milk. Small Ruminant Research. 184: 106015. doi: 10.1016/j.smallrumres.2019.11.001

Othmane MH, Carriedo JA, De la Fuente Crespo LF, San Primitivo F 2002. An individual laboratory cheese-making method for selection in dairy ewes. Small Ruminant Research. 45: 67-73. doi: 10.1016/S0921-4488(02)00079-2

Pan Z, Ye A, Fraser K, Dave A, Singh H 2023. Heat stability of sheep's skim milk: Aggregation and interaction of proteins. International Dairy Journal. 144: 105689. doi: 10.1016/j.idairyj.2023.105689

Park Y, Juárez M, Ramos M, Haenlein G 2007. Physico-chemical characteristics of goat and sheep milk. Small Ruminant Research. 68: 88-113. doi: 10.1016/j.smallrumres.2006.09.013

Pascual-Alonso M, Miranda-de la Lama GC, Aguayo-Ulloa L, Villarroel M, Alierta S, Maria G 2014. Influence of coat colour on Chamarita sheep phenotypes, behaviour, welfare and performances. Animal Genetic Resources. 54: 179-184. doi: 10.1017/S2078633614000010

Pazzola M, Dettori ML, Cipolat-Gotet C, Cecchinato A, Bittante G, Vacca GM 2014. Phenotypic factors affecting coagulation properties of milk from Sarda ewes. Journal of Dairy Science. 97: 7247-7257. doi: 10.3168/jds.2014-8138

Pelayo R, Gutiérrez-Gil B, Garzón A, Esteban-Blanco C, Marina H, Arranz J 2021. Estimation of genetic parameters for cheese-making traits in Spanish Churra sheep. Journal of Dairy Science. 104: 3250-3260. doi: 10.3168/jds.2020-19387

Poulsen N, Buitenhuis A, Larsen L 2015. Phenotypic and genetic associations of milk traits with milk coagulation properties. Journal of Dairy Science. 98: 2079-2087. doi: 10.3168/jds.2014-7944

Puledda A, Gaspa G, Manca MG, Serdino J, Urgeghe PP, Dimauro C, Negrini R, Macciotta NPP 2017. Estimates of heritability and genetic correlations for milk coagulation properties and individual laboratory cheese yield in Sarda ewes. Animal. 11: 920-928. doi: 10.1017/S1751731116002147

Raynal-Ljutovac K, Pirisi A, De Cremoux R, Gonzalo C 2007. Somatic cells of goat and sheep milk: Analytical, sanitary, productive and technological aspects. Small Ruminant Research. 68: 126-144.

Sánchez-Mayor M, Pong-Wong R, Gutiérrez-Gil B, Garzón A, De la Fuente L, Arranz J 2019. Phenotypic and genetic parameter estimates of cheese-making traits and their relationships with milk production, composition and functional traits in Spanish Assaf sheep. Livestock Science. 228: 76-83. doi: 10.1016/j.livsci.2019.08.004

Sanjayaranj I, Lopez-Villalobos N, Blair HT, Janssen PW, Holroyd SE, MacGibbon AK 2023. A Study of Milk Composition and Coagulation Properties of Holstein-Friesian, Jersey, and Their Cross Milked Once or Twice a Day. Dairy. 4: 167-179. doi: 10.3390/dairy4010012

Scholtens MR 2016. Genetic evaluation of milk traits, live weight, somatic cell score, and litter size at birth, and development of a selection index for dairy sheep : a dissertation presented in partial fulfillment of the requirements for the degree of Master of Science at Massey University, Palmerston North, New Zealand. Unpublished Thesis, Massey University.

Seborg DE, Edgar TF, Mellichamp DA, Doyle III FJ 2016. Process dynamics and control, John Wiley and Sons.

Sevi A, Albenzio M, Marino R, Santillo A, Muscio A 2004. Effects of lambing season and stage of lactation on ewe milk quality. Small Ruminant Research. 51: 251-259. doi: 10.1016/S0921-4488(03)00196-2

Timlin M, Tobin JT, Brodkorb A, Murphy EG, Dillon P, Hennessy D, O'Donovan M, Pierce KM, O'Callaghan TF. 2021. The impact of seasonality in pasture-based production systems on milk composition and functionality. Foods. 10: 607.

Vacca GM, Cipolat-Gotet C, Paschino P, Casu S, Usai MG, Bittante G, Pazzola M 2019. Variation of milk technological properties in sheep milk: relationships among composition, coagulation and cheese–making traits. International Dairy Journal. 97: 5-14. doi: 10.1016/j.idairyj.2019.05.002

Chapter 5

Effect of protein polymorphisms on milk composition, coagulation properties, and protein profile in dairy sheep

This chapter has been published elsewhere. It has been reformatted and presented here with permission:

Marshall AC, Vigolo V, De Marchi M, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2025. Effect of protein polymorphisms on milk composition, coagulation properties, and protein profile in dairy sheep. International Dairy Journal. 160: 106102. doi: https://doi.org/10.1016/j.idairyj.2024.106102

The findings of this chapter were presented orally in Session 44 ‘Genetics of food quality’ at the 75th European Association for Animal Production (EAAP) Conference, from 1-5 September 2024, in Florence, Italy.

Abstract

The objective of this study was to quantify the effect of protein polymorphisms on milk composition, coagulation properties, and protein profile in dairy sheep from a New Zealand flock. A total of 470 test-day records, from 147 lactating ewes, were used in the statistical analyses. Protein polymorphisms observed in the RP-HPLC were self-named for purposes of the present study. Data were analysed using a mixed linear model, including the fixed effects of ewe age, litter size, coat colour, and stage of lactation, and, as a covariate, deviation from the median lambing date of the flock. The effects of protein polymorphisms were added to the model, one at a time. Protein polymorphisms were significantly ($p < 0.05$) associated with milk composition and protein profile. Polymorphism of β-lactoglobulin was significantly associated with milk heat stability, being AB type more heat stable than AA. The other processability traits were not significantly affected by protein polymorphisms. Further studies are required to confirm the protein variants and the properties of individual protein polymorphisms.

Keywords

Sheep milk; Dairy sheep; Milk composition; Protein profile; Milk coagulation; Heat stability.

5.1 Introduction

Milk casein and whey proteins occur in several different phenotypes, also called protein polymorphisms. These are determined either by biochemical modifications in the proteins, such as alterations in the amino acid sequence, or by differences in phosphorylation levels, variations in the degree of glycosylation, and differences in chain lengths (Martin et al. 2013). The protein polymorphisms are a result of genetic polymorphisms (single nucleotide polymorphisms, SNPs), mutations in the DNA that result in the presence of more than one type of allele in a locus (Moioli et al. 1998), and a result of post-translational modifications, which are also influenced by genetics and other factors (Fang et al. 2016).

Polymorphisms of casein and whey proteins have been linked both directly and indirectly (due to changes in the proportion of protein fractions) with milk coagulation properties (MCP) in cows, sheep, and goats (Frederiksen et al. 2011; Ketto et al. 2019; Piredda et al. 1993; Garzon and Martinez 1992; Zhang et al. 2023). With the advancement of molecular

genetics, this area remains under active investigation. Furthermore, protein polymorphisms may affect human health (Ali et al. 2022). Hence, there is potential to incorporate genetic variants of proteins that are favorable for milk technological properties or human health in animal breeding programs.

Different laboratory techniques are available for determining protein polymorphisms (Amigo et al. 2000; Bonfatti et al. 2014). High-performance liquid chromatography (HPLC) offers the benefit of identification of silent protein variants, with a shorter total analysis and preparation time compared to alternative methods, allowing for routine analysis of many individual milk samples. However, reports on protein polymorphism using HPLC applied to ovine milk are scarce (De Pascale et al. 2022; Moatsou et al 2004; Trujillo et al. 2000), while it is quite usual for cow milk (Vigolo et al 2022).

In ovine milk, several phenotypes for caseins and whey proteins have been reported, but amino acid sequences have been determined only for some variants (Richardson and Mercier 1979; Ferranti et al. 1995; Chianese et al. 2007; Picariello et al. 2009). In addition, there is still no official protein nomenclature report for sheep milk like there is for cow milk (Farrell Jr et al., 2004). It is possible to confirm the genetic variants of milk proteins with PCR-RFLP, and the polymorphisms of α-lactalbumin (α-LA); β-lactoglobulin (β-LG); and α_{s1}-, α_{s2}-, β-, and κ-casein (α_{s1}-, α_{s2}-, β-, and κ-CN) proteins have been widely associated with the genes LALBA, PAEP, CSN1S1, CSN1S2, CSN2, and CSN3, respectively (Suárez-Vega et al. 2017).

This study aimed to investigate the effect of self-named polymorphisms of casein and whey proteins observed from HPLC of sheep milk on milk composition, protein composition, coagulation traits, and heat stability, and therefore highlight the need for additional research and analytical advancements in this field concerning sheep milk. Further genomic studies are necessary to confirm the genetic variants of the different protein polymorphisms determined by reverse-phase (RP)-HPLC chromatograms as commercial ovine milk protein standards are unavailable. Secondarily, this study investigated the associations between protein composition (quantity of protein fractions) and milk processability.

5.2 Materials and methods

Ethics approval was obtained for this study (Massey University Animal Ethics Committee Protocol 21/45). A total of 470 herd-test records were obtained from 147 ewes at 50 to 182 days of lactation, milked once a day (November 2021- January 2022). The ewes belonged to a small flock located in Masterton, New Zealand. The farm operates on a pasture-based system with low supplementation. Milk collection started after the full weaning of lambs at around four weeks of age. Description of the animals and the farm has been previously reported (Marshall et al. 2023, Chapter 3). The development of the breed started over 27 years ago using mainly East Friesian genetics.

Each ewe had a minimum of two records (Appendix 2 Table 1**)**. The milk yield of individual ewes was manually recorded from the total volume taken from individual test buckets, and a representative milk sample was taken for compositional, processability, and protein profile analyses. The milk samples were immediately refrigerated for transportation to Palmerston North within four hours of collection and had sodium azide added upon arrival (at a final concentration of 0.025%). The samples were kept refrigerated for compositional and processability analyses. The aliquot for protein profile analyses was kept frozen (from -20°C to -80°C) and was sent to the laboratories of the University of Padova (Legnaro, Italy).

5.2.1 Milk composition

An aliquot was analyzed using a Combi FOSS FT6000 instrument (Foss Analytics). The milk composition analyses included fat (%), protein (%), lactose (%), and somatic cell count (SCC, cells/mL). The analyses for casein (%) and urea (mg/100mL) were performed using a Fourier-transform Infrared (FTIR) milk analyser MilkoScan FT6000 (Foss Analytics).

Milk samples were submitted to a contract laboratory (Massey Nutrition Lab) for analysis of total calcium content (mg/100 mL) using the Arsenazo III method (Randox reagent kit Ca8309) and the RX Daytona Plus clinical analyser.

5.2.2 Milk processability

Measures of milk processability traits were described in Marshall et al. (2024, Chapter 4). The traditional MCP were obtained using a Formagraph instrument (Foss Analytics). The parameters obtained from the Formagraph included rennet coagulation time (RCT, min), time to reach curd firmness of 20 mm (K20, min), and curd firmness at 30 min (A30, mm) (McMahon and Brown, 1982).

Milk pH was measured at 31°C, using a calibrated pH meter (EcoScan Model pH5) on the same day as MCP were obtained.

A smaller set of a total of 315 samples, due to feasibility reasons, was processed for measurement of individual laboratory cheese yield (ILCY), as per Othmane et al. (2002). Individual laboratory cheese yield (%) was the relative weight of the centrifuge residue (g) on the initial weighed milk (g).

The heat coagulation time (HCT, min) of whole raw milk was defined as the time of milk coagulation after submerging the sample in an oil bath set at 140°C, as described by Cole and Tarassuk (1946).

5.2.3 Determination of proteins

The protein composition of the individual sheep milk samples was performed through RP-HPLC. The RP-HPLC equipment consisted of an Agilent 1260 Infinity II LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump (Agilent 1260 Infinity II, G7111B) and a diode array detector (Agilent 1260 Infinity II, G7115A). Protein separations were performed on a reversed-phase analytical column C8 (Zorbax 300SB-C8 RP, Agilent Technologies) with silica-based packaging (3.5 μm, 300 Å, 150 mm x 4.6 mm i.d.). A Security Guard Cartridge System (300SB-C8 Guard Cartridges 4.6 mm x 12.5 mm, 4/PK, Agilent Technologies), was used as pre-column.

The sample preparation step followed the method proposed by Bobe et al. (1998). Frozen sheep milk aliquots (500 μL) were thawed at room temperature and treated with an aqueous solution of guanidine (Gdn) HCl (6 M Gdn-HCl, 0.1 M BisTris buffer, 5.37 mM sodium citrate, and 19.5 mM dithiothreitol) in a 1:1 ratio (v/v). Each milk sample was vortexed for 10 s, incubated at room temperature for 1 hour to promote protein solubilization, and centrifuged for 10 min at room temperature at 13,000 x g for fat

separation. After centrifugation, the fat layer was discarded, and the remaining solubilized sample was diluted with a solution containing 4.5 M GdnHCl diluted in a solvent mixture consisting of water, acetonitrile, and trifluoroacetic acid (100:900:1; v/v/v), in a 1:3 ratio (v/v). Gradient elution was carried out with a mixture of solvent A (0.1% trifluoroacetic acid in water) and solvent B (0.1% trifluoroacetic acid in acetonitrile). The separation of milk protein fractions and the chromatographic conditions were described in Bonfatti et al. (2008) and Vigolo et al. (2022), respectively. External standards of κ-CN, α-CN, β-CN, α-LA, β-LG A, and β-LG B (Merck KGaA) of the highest purity available were employed for instrument calibration. Agilent OpenLab 2 CDS software (Agilent Technologies) was used for data acquisition and analysis.

The identification of separated protein fractions was achieved by comparing the elution times with those reported by Trujillo et al. (2000). In particular, the elution times and the characteristics of the corresponding peaks of κ-CN, α_{s1}-CN, α_{s2}-CN, β-CN, α-LA, and β-LG were comparable with those reported by Trujillo et al. (2000). Moreover, separate analysis of whey fractions in a smaller set of samples confirmed its distinct elution from the casein fractions. Finally, the results were expressed as area (mAU) underneath the peaks of κ-CN, α_{s1}-CN, α_{s2}-CN, β-CN, α-LA, and β-LG, which represents the quantity of each protein fraction. A strong linear relationship ($r^2 > 0.90$) was observed between protein concentration in milk (g/L) and combined peak areas of protein fractions (total area), which has also been confirmed previously by Bonfatti et al. (2008). The percentage of each protein in total protein (% TP) was calculated as the area of the respective fraction divided by the total area. Additionally, the method allowed for the observation of protein polymorphisms, with self-named nomenclature being given based on peak characteristics in the chromatogram. Further genomic studies are required for confirmation of protein genetic variant (allele genotype discrimination).

5.2.4 Statistical analyses

Descriptive statistics (mean, standard deviation, minimum and maximum values, and coefficient of variation) for milk yield, milk composition, protein composition, milk processability (RCT, K20, A30, ILCY, HCT), pH, and somatic cell score (SCS) were obtained in SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). Analyses of variances were performed using the MIXED procedure with a linear model that included the fixed effects of ewe coat colour as an indicator of genetic variety within the breed (categorical variable with two levels: black or white), litter size (categorical variable with two levels: 1 lamb or 2 lambs and greater), ewe age (categorical variable with four levels: 1, 2, 3, and 4 years and older), and stage of lactation as a categorical variable with three levels: 1, 2, and 3, representing the different ranges of days in milk (date of test – date of lambing) from ≥50 and <95, ≥95 and <140, ≥140 and ≤182, respectively.

The fixed effects of self-named protein polymorphisms were included in the model, one at a time, these included the effect of aggregated caseins (categorical variable with two levels: AA/AA/AA or AB/AB/AB), α-LA (categorical variable with three levels: AA, AB, or BB), and β-LG (categorical variable with two levels: AA or AB). Polymorphisms that represented less than 7% of the population were not considered as an effect. As a covariate, deviation from the median lambing date of the flock (DMLD, days) was added to the model. Random effects included a random effect of ewe and random residual error. Least squares means and standard errors for each class of the fixed effect of self-named protein polymorphism were obtained and used for mean comparisons using Fisher`s least significant difference. The GLM procedure was used to investigate partial correlations between the content of protein fractions (% TP) and the processability traits, using the same animal model, as described above.

5.3 Results

5.3.1 Individual chromatograms

The RP-HPLC chromatograms from two individual milk samples are provided in Figure 5.1, to illustrate the self-named protein polymorphisms observed in the flock, and included as fixed effects in the model. In the present study, κ-CN was monomorphic, and the polymorphisms of casein proteins (α_{s1}-, α_{s2}-, β-CN) seemed to be inherited together as a haplotype. It was observed that α_{s1}-CN occurred in duplicate, for some ewes, with two very distinct fractions that eluted separately (19 min vs 20 min), these were named AB and are suggested to be a heterozygous form of α_{s1}-CN. The ewes with only one fraction of α_{s1}-CN are likely to be homozygous for α_{s1}-CN, and this form was named AA (20 min). Both heterozygous (AB) and homozygous (AA) forms of α_{s1}-CN had a minor peak eluting after a major peak. The least frequent form (named BB) was the single fraction (also with a minor peak) that eluted earlier than AA (19 min).

The α_{s2}-CN and β-CN were also present as a single peak (named AA), with two distinct peaks (named AB), or as a single peak (named BB) that eluted earlier than AA. The given polymorphism names do not necessarily reflect heterozygous or homozygous forms of the same genetic variant, and they could be distinct genetic variants of different phosphorylation levels.

The minor peaks observed for α_{s1}- and α_{s2}-CN, in the same individuals, were sometimes with a broad rather than a sharp shape, and of heights that fluctuated throughout the lactation/season. For α_{s1}-CN there was a clear trend, the minor peaks became wider and less sharp with the advancement of the lactation/season. In addition, the double peaks of β-CN AB became sharper and taller with the advancement of the season or lactation.

For the whey proteins, α-LA and β-LG also occurred as either a double peak (AB), a single peak (AA) of later elution time, or a single peak of earlier elution time (BB). For α-LA and β-LG less variation is noted in the literature, and the observable variants of β-LG are either A or B, with C being uncommon.

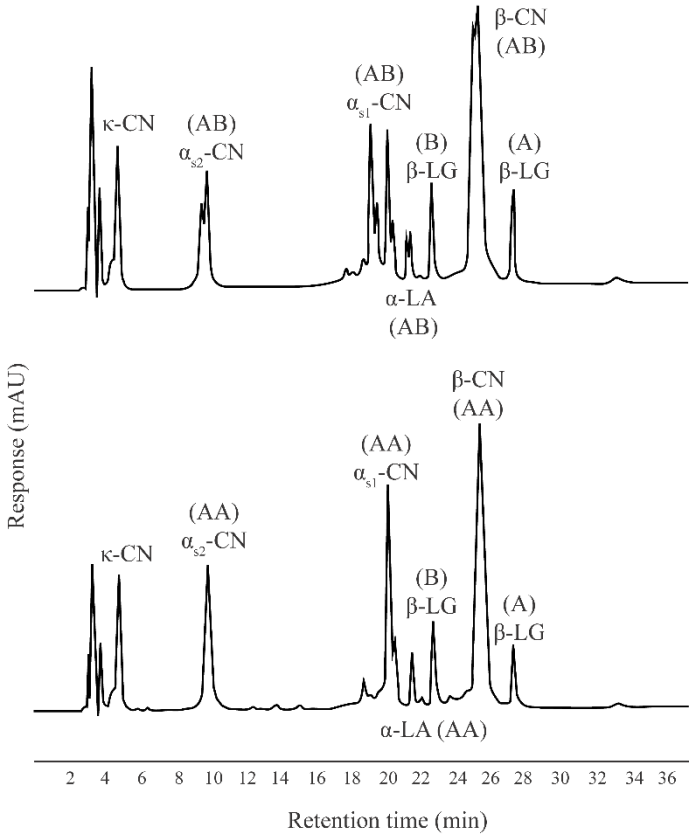


Figure 5.1. Two individual RP-HPLC chromatograms illustrate all the possible self-named milk protein polymorphisms observed in the studied dairy sheep population. The protein fractions are represented by the response or peak area (measured as the intensity of absorbance, mAU) plotted as a function of retention time (min). Top chromatogram: κ-casein (monomorphic, approx. retention time between 4-5 min), followed by one fraction of α_{s2}-casein with double peak, named AB (9-10 min); two fractions of α_{s1}-casein with double peak, named AB (19-21 min); α-lactalbumin with two fractions, named AB (21-22 min); β-lactoglobulin named B (22-23 min); β-casein with a double peak, named AB (25-26 min); and β-lactoglobulin named A (27-28 min). Bottom chromatogram: κ-casein (monomorphic), followed by one fraction of α_{s2}-casein with a single peak, named AA (9-10 min); one fraction of α_{s1}-casein with a double peak, named AA (19-21 min); α-lactalbumin with one fraction, named AA (21-22 min); β-lactoglobulin named B (22-23 min); β-casein with a single peak, named AA (25-26 min); and β-lactoglobulin named A (27-28 min).

5.3.2 Frequency of polymorphisms

The frequencies of the milk protein polymorphisms observed in the studied flock are presented in Appendix 2 Table 2.

5.3.3 Average protein composition

Descriptive statistics of milk yield and composition, protein composition (% TP), SCS, milk coagulation, cheese yield, heat coagulation time, and milk pH are summarised in Table 5.1.

The κ-CN, α_{s1}-CN, α_{s2}-CN, and β-CN constituted on average 7.3, 27.6, 12.8, and 40.1 % TP, respectively. The α-LA and β-LG constituted 1.9% and 10.4% TP, respectively. Coefficients of variation were largest for the content of α-LA (32%), followed by the content of κ-CN (18%). The lowest coefficient of variation was that of β-CN, with only 5% of variation.

Table 5.1. Means, standard deviations (SD), minimum and maximum values, and coefficients of variation (CV) for milk production, milk composition, protein composition, SCS, milk coagulation properties, cheese yield, heat coagulation time, and milk pH of dairy sheep milked once-a-day during the 2021-2022 production season.

Trait[1]	N	Mean	SD	Minimum	Maximum	CV (%)
Milk yield (L/day)	470	0.57	0.25	0.10	1.80	44
Milk composition						
Fat (%)	470	6.32	1.26	1.87	11.24	20
Protein (%)	470	5.52	0.68	4.09	8.80	12
Lactose (%)	470	4.72	0.27	3.66	5.34	6
Casein (%)	470	4.21	0.50	3.08	6.28	12
Casein:Protein	470	0.77	0.02	0.54	0.82	3
Calcium (mg/100 mL)	470	185.5	25.3	104.6	269.4	14
Urea (mg/100 mL)	470	39.64	7.61	9.39	63.13	19
SCS (Log_2SCC)	470	16.16	1.96	9.97	23.83	12
SCC × 10^3 (cells/mL)	470	275	1090	1	14906	400
Protein composition (% TP)						
κ-casein	470	7.28	1.30	2.60	13.80	18
α_{s1}-casein	470	27.58	1.78	20.60	32.80	6

α_{s2}-casein	470	12.78	1.58	5.90	17.80	12
β-casein	470	40.06	1.96	32.50	45.50	5
α-lactalbumin	470	1.94	0.61	0.09	4.60	31
β-lactoglobulin	470	10.36	1.25	6.20	14.30	12
Milk coagulation properties						
RCT (min)	451	13.3	3.2	6.86	26.0	24
K20 (min)	447	2.7	1.1	1.30	10.2	41
A30 (mm)	451	53.3	9.5	11.60	75.4	18
ILCY (%)	315	44.20	8.97	23.4	78.8	20
HCT (min)	295	1.41	0.81	0.07	5.00	57
pH	470	6.59	0.09	6.34	6.90	1

[1] Casein:Protein= ratio of casein content to protein content, calculated as casein (%) divided by protein (%); SCS=somatic cell score (Log_2 SCC); SCC= somatic cell count; TP=total protein; RCT= rennet coagulation time; K20= time to reach curd firmness of 20 mm; A30= curd firmness at 30 min post rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time.

5.3.4 Effects of animal factors

The F-values for the effects of animal factors on the milk traits investigated are presented in Appendix 2 Table 3. The least squares means of protein composition for different classes of ewe coat colour, litter size, age, and different stages of lactation are presented in Appendix 2 Table 4. The detailed investigation of the effects of physiological and environmental factors on the protein composition was beyond the scope of the present study. These were included in the model to obtain an unbiased estimation of the effects of protein polymorphisms.

5.3.5 Effects of protein polymorphisms

The F-values for the effects of protein polymorphism are presented in Table 5.2. The least squares means of milk composition, processability, and protein composition, for each protein polymorphism, corrected for the fixed effects, are presented in Tables 5.3−5.5.

Table 5.2. F-values for effects of self-named protein polymorphisms on milk yield and composition, contents of calcium and urea, SCS, protein composition, milk coagulation properties, cheese yield, heat coagulation time, and milk pH of dairy sheep milked once-a-day during the 2021-2022 production season.

Trait[1]	Casein polymorphism (α_{s1}/α_{s2}/β-casein)	α-lactalbumin polymorphism	β-lactoglobulin polymorphism
Milk yield (L/day)	0.79	0.94	0.18
Fat (%)	2.53	2.63	2.62
Protein (%)	2.43	3.38*	0.46
Lactose (%)	2.60	2.89	0.21
Casein (%)	1.35	4.58**	0.30
Casein:Protein	1.87	0.95	0.28
Calcium (mg/100 mL)	0.14	0.63	0.12
Urea (mg/100 mL)	2.03	1.44	1.80
SCS (Log_2SCC)	0.19	0.84	3.70
Protein composition (% TP)			
κ-casein	1.07	0.09	0.32
α_{s1}-casein	41.50***	1.22	1.45
α_{s2}-casein	60.40***	1.20	0.01
β-casein	4.00*	1.08	0.44
α-lactalbumin	0.02	15.60***	1.08
β-lactoglobulin	8.00**	0.67	0.44
Milk coagulation properties			
RCT (min)	0.26	0.43	1.70
K20 (min)	0.01	1.62	0.12
A30 (mm)	0.19	1.16	0.43
ILCY (%)	0.01	0.65	0.60
HCT (min)	3.26	0.08	5.02*
pH	2.96	0.70	3.50

[1] Casein:Protein= ratio of casein to protein, calculated as casein (%) divided by protein (%); SCS=somatic cell score (Log_2 SCC); SCC= somatic cell count; TP= total protein; RCT= rennet coagulation time; K20= time to reach curd firmness of 20 mm; A30= curd firmness at 30 min post rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time.
Statistical significance is given as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 5.3. Least squares means (± standard errors) of milk composition (percentages of fat, protein, lactose, and casein), and SCS, for the different self-named protein polymorphisms.

Protein[1]	Polymorphism[2]	Fat (%)	Protein (%)	Lactose (%)	Casein (%)	SCS (Log_2SCC)
Casein ($\alpha_{s1}/\alpha_{s2}/\beta$-CN)	AA/AA/AA	6.26 ± 0.09	5.50 ± 0.05	4.42 ± 0.02	4.17 ± 0.04	16.29 ± 0.17
	AB/AB/AB	6.06 ± 0.13	5.38 ± 0.07	4.77 ± 0.02	4.10 ± 0.05	16.39 ± 0.24
Whey fractions						
α-LA	AA	6.19 ± 0.11	5.37^{b} ± 0.06	4.77 ± 0.02	4.06^{b} ± 0.05	16.20 ± 0.21
	AB	6.09 ± 0.11	5.50^{ab} ± 0.06	4.72 ± 0.02	4.18^{a} ± 0.05	16.49 ± 0.21
	BB	6.44 ± 0.14	5.61^{a} ± 0.08	4.69 ± 0.03	4.27^{a} ± 0.06	16.25 ± 0.25
β-LG	AA	6.16 ± 0.09	5.46 ± 0.05	4.74 ± 0.02	4.14 ± 0.04	16.44 ± 0.17
	AB	6.38 ± 0.13	5.51 ± 0.07	4.72 ± 0.03	4.18 ± 0.06	15.97 ± 0.24

[1] $\alpha_{s1}/\alpha_{s2}/\beta$-CN= aggregate (haplotype) of α_{s1}-casein, α_{s2}-casein, and β-casein proteins; α-LA= α-lactalbumin; β-LG= β-lactoglobulin; SCS= somatic cell score calculated as Log_2SCC; SCC= somatic cell count.

[2] Nomenclature was self-given to the protein polymorphisms in this population of dairy sheep, as they were being identified in the RP-HPLC chromatograms. Genomic studies and other laboratorial techniques for the description of protein structure will enable the confirmation of any protein genetic variants previously reported in the literature.

[a, b, c] Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Table 5.4. Least squares means (± standard errors) of milk coagulation properties, heat coagulation time, and milk pH, for the different self-named protein polymorphisms.

Protein[1]	Polymorphism[2]	RCT (min)	K20 (min)	A30 (mm)	HCT (min)	pH
Casein						
(α_{s1}/α_{s2}/β-CN)	AA/AA/AA	14.63 ± 0.51	3.23 ± 0.25	52.00 ± 1.30	1.68 ± 0.09	6.59 ± 0.01
	AB/AB/AB	14.28 ± 0.71	3.20 ± 0.35	51.24 ± 1.82	1.86 ± 0.11	6.61 ± 0.01
Whey fractions						
α-LA	AA	14.41 ± 0.62	3.18 ± 0.30	51.69 ± 1.60	1.74 ± 0.10	6.60 ± 0.01
	AB	14.91 ± 0.63	3.53 ± 0.31	50.64 ± 1.61	1.70 ± 0.10	6.59 ± 0.01
	BB	14.22 ± 0.76	2.82 ± 0.37	53.85 ± 1.95	1.73 ± 0.12	6.60 ± 0.01
β-LG	AA	14.28 ± 0.52	3.19 ± 0.26	52.16 ± 1.34	1.67^{b} ± 0.09	6.59 ± 0.01
	AB	15.52 ± 0.72	3.31 ± 0.36	50.90 ± 1.86	1.91^{a} ± 0.11	6.61 ± 0.01

[1] RCT= rennet coagulation time; K20= time to reach curd firmness of 20 mm; A30= curd firmness at 30 minutes post rennet addition; HCT= heat coagulation time; α_{s1}/α_{s2}/β-CN= aggregate (haplotype) of α_{s1}-casein, α_{s2}-casein, and β-casein proteins; α-LA= α-lactalbumin; β-LG= β-lactoglobulin.

[2] Nomenclature was self-given to the protein polymorphisms in this population of dairy sheep, as they were being identified in the RP-HPLC chromatograms. Genomic studies and other laboratorial techniques for the description of protein structure will enable the confirmation of any protein genetic variants previously reported in the literature.

[a, b, c] Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Table 5.5. Least squares means (± standard errors) of protein composition (percentages of κ-casein, α_{s1}-casein, α_{s2}-casein, β-casein, α-lactalbumin, and β-lactoglobulin, in total protein) for the different self-named protein polymorphisms.

Protein[1]	Polymorphism[2]	κ-CN (% TP)	α_{s1}-CN (% TP)	α_{s2}-CN (% TP)	β-CN (% TP)	α-LA (% TP)	β-LG (% TP)
Casein							
(α_{s1}/α_{s2}/β-CN)	AA/AA/AA	7.73 ± 0.10	26.56^{b} ± 0.17	13.61^{a} ± 0.15	39.88^{a} ± 0.18	2.08 ± 0.05	10.12^{b} ± 0.11
	AB/AB/AB	7.88 ± 0.14	28.06^{a} ± 0.24	12.02^{b} ± 0.21	39.39^{b} ± 0.25	2.09 ± 0.08	10.55^{a} ± 0.15
Whey fractions							
α-LA	AA	7.74 ± 0.13	26.87 ± 0.24	13.16 ± 0.22	39.69 ± 0.22	2.29^{a} ± 0.06	10.23 ± 0.14
	AB	7.76 ± 0.13	27.16 ± 0.24	13.09 ± 0.22	39.66 ± 0.22	2.02^{b} ± 0.06	10.30 ± 0.14
	BB	7.82 ± 0.16	26.69 ± 0.29	13.53 ± 0.27	40.07 ± 0.27	1.80^{c} ± 0.07	10.09 ± 0.17
β-LG	AA	7.74 ± 0.11	26.84 ± 0.20	13.23 ± 0.18	39.81 ± 0.18	2.10 ± 0.05	10.25 ± 0.11
	AB	7.83 ± 0.15	27.18 ± 0.28	13.20 ± 0.25	39.64 ± 0.26	2.02 ± 0.08	10.14 ± 0.16

[1] α_{s1}/α_{s2}/β-CN= aggregate (haplotype) of α_{s1}-casein, α_{s2}-casein, and β-casein proteins; α-LA= α-lactalbumin; β-LG= β-lactoglobulin; κ-CN= κ-casein; α_{s1}-CN= α_{s1}-casein; α_{s2}-CN= α_{s2}-casein; β-CN= β-casein; TP= total protein.

[2] Nomenclature was self-given to the protein polymorphisms in this population of dairy sheep, as they were being identified in the RP-HPLC chromatograms. Genomic studies and other laboratorial techniques for the description of protein structure will enable the confirmation of any protein genetic variants previously reported in the literature.

[a, b, c] Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

No effect of casein polymorphisms on milk production or milk composition was observed in the present study for this flock of dairy sheep. The only protein polymorphism with a significant effect ($p < 0.05$) on the gross milk composition of ewes was that of α-LA. The polymorphism named BB of α-LA was superior for contents of protein and casein in milk, followed by AB, and AA (BB>AB>AA). Polymorphism of β-LG almost had a significant effect on SCS, with polymorphism AB tending to have lower SCS.

The casein polymorphisms (combined α_{s1}-/α_{s2}-/β-CN) significantly affected the percentages of α_{s1}-, α_{s2}-, β-CN, and β-LG in TP. The polymorphism of α-LA significantly affected its concentration in TP. The polymorphism of β-LG did not significantly affect any of the protein concentrations. The concentration of κ-CN was not affected by any of the protein polymorphisms.

The only trait related to milk processability that was significantly affected by protein polymorphism was HCT. Polymorphism B of β-LG significantly increased milk heat stability, with AB milk coagulating 0.24 min (14 s) later than AA milk at 140°C. Noteworthy, β-LG AB milk also tended to have a higher pH than AA milk, but this difference was not significant. The casein polymorphisms did not affect any traits relevant to cheese-making (RCT, A30, K20, and ILCY).

5.3.6 Correlation between protein fractions and processability

The partial correlation coefficients between the content of protein fractions (% TP) and processability traits are presented in Table 5.6.

Most notable correlations were found between contents of κ-, α_{s1}-, β-CN, α-LA %, and ILCY. Increased κ- and α_{s1}-CN % were correlated with increased cheese yield, and the opposite was found for β-CN and α-LA %. Also, of notable importance, was the higher κ-CN % correlated with increased A30 and HCT. Additionally, higher α-LA % correlated with increased HCT, whereas higher β-LG % correlated with decreased HCT.

Table 5.6. Partial correlation coefficients between the content of protein fractions (percentages of κ-casein, α_{s1}-casein, α_{s2}-casein, β-casein, α-lactalbumin, and β-lactoglobulin, in total protein) and processability traits (RCT, K20, A30, ILCY, and HCT).

	Content of protein fractions (% in total protein)[2]					
Processability traits[1]	κ-CN	α_{s1}-CN	α_{s2}-CN	β-CN	β-LG	α-LA
RCT (min)	-0.04	0.02	-0.04	0.08	-0.03	-0.12
	(0.46)	(0.69)	(0.47)	(0.18)	(0.66)	(0.05)
K20 (min)	-0.13	0.00	-0.11	0.13	0.02	-0.02
	(0.04)	(0.94)	(0.07)	(0.03)	(0.71)	(0.70)
A30 (mm)	0.17	-0.04	0.12	-0.10	-0.04	0.02
	(0.01)	(0.48)	(0.05)	(0.08)	(0.45)	(0.80)
ILCY (%)	0.39	0.29	-0.08	-0.36	0.07	-0.27
	(<0.001)	(<0.001)	(0.17)	(<0.001)	(0.27)	(<0.001)
HCT (min)	0.16	-0.12	0.00	0.07	-0.14	0.17
	(0.01)	(0.05)	(0.94)	(0.28)	(0.02)	(0.01)

[1]RCT= rennet coagulation time; K20= time to reach curd firmness of 20 mm; A30= curd firmness at 30 minutes post rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time.

[2]κ-CN= κ-casein; α_{s1}-CN= α_{s1}-casein; α_{s2}-CN= α_{s2}-casein; β-CN= β-casein; β-LG= β-lactoglobulin; α-LA= α-lactalbumin.

5.4 Discussion

5.4.1 Average protein composition

To our knowledge, there are only a few reports available on the quantity of sheep milk casein fractions and they are contradictory, especially regarding the predominance of either α_{s1}- or α_{s2}-CN. Bramanti et al. (2003) also reported a higher concentration of α_{s1}-CN than of α_{s2}-CN fraction, in agreement with the present study. Protein composition was similar to the report of Moatsou et al. (2004) for Greek ewes, but different from the reports for Polish mountain (Kawęcka and Radkowska 2023) and Romanian ewes (Pelmus et al. 2012). Differences might be largely attributed to the distinct sheep breeds and farming systems as feed is also reported to influence milk protein composition (Kawęcka and Radkowska 2023).

5.4.2 Protein polymorphisms

Previous studies report κ-CN as monomorphic in dairy sheep, in agreement with the present study (Suárez-Vega et al. 2017). In East-Friesian Dairy sheep, sequencing of CSN3 mRNA led to the identification of a new synonymous SNP (Giambra et al. 2014), which does not affect protein sequence. In Merino sheep, a microsatellite in intron 3 of ovine CSN3 was identified with five alleles (Corral et al. 2010), and neutral amino acid exchange has also been reported (Ceriotti et al. 2004).

Others have also considered the polymorphisms of casein proteins (α_{s1}-, α_{s2}-, β-CN) as a haplotype that is inherited together (Gai et al. 2021; Sacchi et al. 2005), in agreement with the findings in our study. This is due to the close location of the casein genes, which are organised in a cluster of less than 250 kb in length.

Also, in agreement with the present study, changes in the shape of double peaks were previously noted by Moatsou et al. (2004) in Greek sheep. This is suggested to be due to post-translational differences and to different genetic expressions of the level of phosphorylation. Other factors were also suggested to affect the level of protein phosphorylation such as the health and age of individuals, and the availability of phosphate (Amigo et al. 2000). In addition, seasonal variation of the level of glycosylation of κ-CN has been noted for grazing dairy cows (Li et al. 2019), and these are worth investigating in dairy sheep.

The use of different HPLC methodologies in different studies (De Pascale et al. 2022; Picariello et al. 2009) impedes comparisons regarding the identification of genetic variants of α_{s1}- and α_{s2}-CNs. β-CN is also known to be highly polymorphic, though less phosphorylated than α_{s1}-CN and α_{s2}-CN (Chessa et al. 2010).

The polymorphisms of α-LA are the least studied among the milk proteins (Selvaggi et al. 2014). Less genetic variation is observed for this protein. Two ovine α-lactalbumin protein patterns (A and B) have been evidenced by starch gel electrophoresis, being the A variant the most common (Amigo et al. 2000; Schmidt and Ebner 1972).

The most studied polymorphisms in sheep are those of β-LG, which have also been investigated by chromatography (El-Zahar et al. 2004; Picariello et al. 2012; Trujillo et al. 2000). Three variants of β-LG have been identified in the literature for dairy sheep (A, B, C), A and B being the most common. Thus, it is very likely that the β-LG variants identified in the present study are A and B.

5.4.3 Effect of protein polymorphisms on milk yield and composition

Protein genotypes of sheep have been previously associated with milk production and composition in other sheep populations (Corral et al. 2010; Dario et al. 2008; Noce et al. 2016; Pirisi et al. 1999; Pirisi et al. 1999; Sallam 2023; Yousefi et al. 2013). However, contradictory results are reported, and this is attributed to breed differences, population size, frequency of distribution of genetic variants, and differences in methods used for statistical analysis. Furthermore, the effect of protein polymorphisms may change throughout lactation, due to differential expression of milk protein genes (Cardona et al. 2016).

There is limited information available in the literature regarding the effect of genetic variants of α-LA on quantitative milk traits (Sallam 2023; Selvaggi et al. 2014). A strong association between α-LA genetic variants with milk protein and fat contents has been reported for Churra sheep (Garcia-Gamez et al. 2012), which aligns in part with the present study. It was suggested that an amino-acid substitution of α-LA would generate a decrease in lactose synthesis and milk osmotic pressure, affecting fat and protein concentrations and early studies have demonstrated the importance of α-LA as a regulator of lactose production and secretion of milk (Stacey et al. 1995). Another study found that

heterozygote ewes of the LALBA gene tended to produce more milk with a greater total solid percentage (Sallam 2023).

Although not significant in the present study, an association between β-LG genotype and SCC in ewes has been previously reported, also with lower SCC for heterozygous ewes (Triantaphyllopoulos et al. 2017). Effects of β-LG genotype on milk production and composition were found by others and the results reported were controversial (Corral et al. 2010; Dario et al. 2008; Ibrahim et al. 2019; Ramos et al. 2009; Yousefi et al. 2013).

Although no significant effects of casein polymorphisms on milk production and composition were found in the present study, which could be a consequence of the small population size, others found casein genotypes to be strongly associated with ewe milk production and composition (Pirisi et al. 1999b; Ramos et al. 2009; Corral et al. 2010; Giambra et al. 2014). In East Friesian and Lacaune ewes from Switzerland and Germany, an α_{s1}-CN variant was associated with higher protein content (Giambra et al. 2014). Also, an interaction effect of CSN2 with CSN1S1 polymorphisms on milk production and composition was discovered in Awassi sheep (Al-Amareen and Jawasreh 2022). In addition, genotypes of the CSN3 gene showed significant influences on the protein content of milk in East Friesian sheep (Giambra et al. 2014).

No studies were found on the association between milk pH and ovine milk protein variants, however, for bovine milk, the pH of skim milk samples has been associated with α_{s1}-CN genetic variants (McLean et al. 1987). The polymorphisms of caseins and β-LG almost had a significant effect on sheep milk pH in the present study.

5.4.4 Effect of protein polymorphisms on protein composition

While numerous studies have explored the association between protein genetic variants and differences in gross milk composition (Corral et al. 2010; Noce et al. 2016; Sallam 2023), there remains a scarcity of research correlating protein genetic variants with protein composition in sheep milk (Nudda et al. 2003). It has been noted that variations in the coagulating properties of milk are predominantly linked to differences in the content of protein fractions rather than being solely influenced by the direct effect of protein genotypes (Bonfatti et al. 2010; Cipolat-Gotet et al. 2018; Wedholm 2008).

For instance, in cow milk, firmer curd was observed with lower proportions of α_{s2}-CN and β-CN or higher ratios of κ-CN (Jõudu et al. 2008). The concentration of κ-CN in the casein fraction is known to largely influence MPC because κ-CN is negatively correlated with casein micelle size. Low content of κ-CN increases the risk of non-coagulation because larger casein micelles are formed, which aggregate slower and form a softer curd (Ford and Grandison 1986; Frederiksen et al. 2011). In agreement with the present study, Bonfatti et al. (2010) reported that RCT was favorably affected by high κ-CN content and percentage of κ-CN to total casein in bovine milk, and A30 increased with increased κ-CN content.

Additionally, milk protein composition can vary due to factors related to the animal physiology such as lactation stage and parity number, which was also noted in the present study, as well as with health status, feeding, and management (Bobe et al. 1998; Frederiksen et al. 2011).

Notably, protein composition has been extensively associated with milk protein genetic variants in dairy cows (Bonfatti et al. 2010; Jõudu et al. 2008; Ketto et al. 2017; McLean et al. 1987), in agreement with the present study where the effects of casein and α-LA polymorphisms on protein composition of sheep milk were significant. However, Nudda et al. (2003) found no significant effect of the ovine β-LG genotype on the contents (g L^{-1}) of β-LG or α-LA, which aligns with our findings.

In addition, in agreement with our study, McLean et al. (1987) noted that cow milk heat stability was positively associated with κ-CN concentrations and negatively correlated with α_{s1}-CN and β-LG concentrations. Also in agreement with our study, a previous study found that increased α-LA (increased α-LA:β-LG ratio) improved resistance to heat-induced coagulation, due to decreased protein-protein interactions, and that β-LG content had a destabilising effect (Crowley et al. 2016).

5.4.5 Effect of β-LG polymorphism on processability

In alignment with the present findings, it has been suggested that the Tyr of β-LG A has a role in the hydrophobic interactions, affecting the stability of micelles, and therefore B should show a higher denaturation onset temperature than ovine A (Amigo et al. 2000).

For bovine milk, β-LG variant B was also considered more thermostable than variant A (Keppler et al. 2014).

It was found that during heat treatment of milk, a complex is formed between κ-CN and β-LG (Dalgleish 1990). The destabilising effect of β-LG at a pH of minimum stability was linked to the ability to increase the hydrophobicity of casein micelles, sensitising casein micelles to heat-induced precipitation of calcium phosphate. However, at a pH of maximum stability, β-LG could chelate calcium (O'Connell and Fox 2001). Hence, the relationship between β-LG variants and the heat stability of milk was found to be dependent on the pH range (Imafidon et al. 1991), as well as milk concentration (McLean et al. 1987), and temperature applied (Jakob and Puhan, 1992). Consequently, conflicting results have emerged in the literature for bovine milk.

Although no significant effect of β-LG polymorphism on the cheesemaking properties was found in the present study on ovine milk, significant effects of β-LG genotypes on MCP have been observed for bovine milk (Bonfatti et al. 2010; Marziali and Ng-Kwai-Hang 1986).

5.4.6 Effect of casein polymorphisms on processability

Despite no significant effects of casein polymorphisms on processability being found in the present study, other studies reported significant associations with renneting properties of milk (Pirisi et al. 1999b; Noce et al. 2016). The lack of the negatively charged phosphate groups, which are the primary binding sites for Ca^{2+}, of an α_{s1}-CN variant was suggested to decrease the association of proteins in solution (Chianese et al. 1996)

Noce et al. (2016) found a particular SNP at the CSN1S1 gene to be associated with RCT, K20, and A30, with the shortest coagulation times and the highest values of curd firmness for heterozygous ewes. Those authors also reported significant associations between the CSN2 genetic variant and K20, which had not been previously investigated in dairy sheep.

While limited studies have explored correlations between ovine casein polymorphisms and the technological properties of milk, extensive research has been conducted in bovine milk (Ketto et al. 2019; Bonfatti et al. 2010). The smaller net charge of a bovine α_{s1}-CN variant contributed to a stronger association (Schmidt 1970), which resulted in a firmer curd, whereas a less hydrophobic variant contributed to a softer curd (Creamer et al. 1982). A

bovine α_{s2}-CN variant was found to be less sensitive to Ca^{2+} due to the reduced number of anionic phosphoryl clusters (Swaisgood 2003). In addition, variants of β-CN were also shown to have a direct effect on RCT, independent of the content of β-CN (Bonfatti et al. 2010).

Additionally, the level of casein phosphorylation is known to affect the buffering capacity of milk, with highly phosphorylated caseins associated with poor rennet and acid coagulation properties (Frederiksen et al. 2011; Ketto et al. 2017). Overall, glycosylation and phosphorylation of caseins can affect processability due to changes in the isoelectric point, molecular weight, hydrophobicity, and net charge of casein micelles (Huppertz et al. 2018), and further work is recommended to establish the effect of glycosylation and phosphorylation degree of the different casein polymorphisms on the coagulation properties of milk from individual sheep.

5.5 Conclusion

Protein polymorphisms were evidenced in the RP-HPLC chromatograms of individual sheep milk samples, despite the small flock size. There was quantitative variability in the protein fractions, especially for the contents of α-LA and κ-CN in TP. The protein polymorphisms were associated with differences in the proportions of protein fractions, and these were correlated to processability traits, especially the content of κ-CN, but also α-LA. Higher κ-CN was associated with overall better processability for cheese-making and heat-stability, and α-LA with better heat stability. There was a significant effect of β-LG polymorphism on milk heat stability, and this could be of great interest to the manufacturers of sheep milk powder and beverages. Validation of the RP-HPLC method for analysis of genetic variants of ovine milk proteins is necessary with the use of additional genomic studies, as the lack of sheep milk protein standards and the lack of studies using combined techniques hinder effective comparisons of chromatograms. Additionally, establishing a nomenclature committee for ovine milk protein identification would be beneficial.

References

Al-Amareen AH., Jawasreh KI. 2022. Single and combined effects of CSN1S1 and CSN2-casein genes on Awassi sheep milk quantity and quality. Veterinary World. 15: 435.

Ali M, Gautam D, Deepika S, Meena AS, Chera J, De S. 2022. The genetic variations in CSN2 gene of Indian sheep breeds affect its protein stability and function. Small Ruminant Research. 207: 106612.

Amigo L, Recio I, Ramos M. 2000. Genetic polymorphism of ovine milk proteins: its influence on technological properties of milk—a review. International Dairy Journal. 10: 135–149.

Bobe G, Beitz DC, Freeman AE, Lindberg GL. 1998. Separation and quantification of bovine milk proteins by reversed-phase high-performance liquid chromatography. Journal of Agricultural and Food Chemistry. 46: 458–463.

Bonfatti V, Grigoletto L, Cecchinato A, Gallo L, Carnier P. 2008. Validation of a new reversed-phase high-performance liquid chromatography method for separation and quantification of bovine milk protein genetic variants. Journal of Chromatography A. 1195: 101–106.

Bonfatti V, Di Martino G, Cecchinato A, Degano L, Carnier P. 2010. Effects of β-κ-casein (CSN2-CSN3) haplotypes, β-lactoglobulin (BLG) genotypes, and detailed protein composition on coagulation properties of individual milk of Simmental cows. Journal of Dairy Science. 93: 3809–3817.

Bonfatti V, Tuzzato M, Chiarot G, Carnier P. 2014. Variation in milk coagulation properties does not affect cheese yield and composition of model cheese. International Dairy Journal. 39: 139–145.

Bramanti E, Sortino C, Onor M, Beni F, Raspi G. 2003. Separation and determination of denatured α_{s1}-, α_{s2}-, β-and κ-caseins by hydrophobic interaction chromatography in cows', ewes' and goats' milk, milk mixtures and cheeses. Journal of Chromatography A. 994: 59-74.

Cardona SJC, Cadavid HC, Corrales JD, Munilla S, Cantet RJ, Rogberg-Muñoz A. 2016. Longitudinal data analysis of polymorphisms in the κ-casein and β-lactoglobulin genes shows differential effects along the trajectory of the lactation curve in tropical dairy goats. Journal of Dairy Science. 99: 7299-7307.

Ceriotti G, Chessa S, Bolla P, Budelli E, Bianchi L, Duranti E, Caroli A. 2004. Single nucleotide polymorphisms in the ovine casein genes detected by polymerase chain reaction-single strand conformation polymorphism. Journal of Dairy Science. 87: 2606-2613.

Chessa S, Rignanese D, Berbenni M, Ceriotti G, Martini M, Pagnacco G, Caroli A. 2010. New genetic polymorphisms within ovine β-and α_{s2}-caseins. Small Ruminant Research. 88: 84-88.

Chianese L, Garro G, Mauriello R, Laezza P, Ferranti P, Addeo F. 1996. Occurrence of five α_{s1}-casein variants in ovine milk. Journal of Dairy Research. 63: 49–59. https://doi.org/10.1017/S0022029900026865

Cipolat-Gotet C, Cecchinato A, Malacarne M, Bittante G, Summer A. 2018. Variations in milk protein fractions affect the efficiency of the cheese-making process. Journal of Dairy Science. 101: 8788–8804. https://doi.org/10.3168/jds.2017-14056

Cole WC, Tarassuk NP. 1946. Heat coagulation of milk. Journal of Dairy Science. 29: 421–429. https://doi.org/10.3168/jds.S0022-0302(46)92441-7

Corral J, Padilla J, Izquierdo M. 2010. Associations between milk protein genetic polymorphisms and milk production traits in Merino sheep breed. Livestock Science. 129: 73–79. https://doi.org/10.1016/j.livsci.2010.01.016

Creamer L, Zoerb H, Olson N, Richardson T. 1982. Surface hydrophobicity of α_{s1}-I, α_{s1}-casein A and B and its implications in cheese structure. Journal of Dairy Science. 65: 902–906. https://doi.org/10.3168/jds.S0022-0302(82)82283-5

Crowley SV, Dowling AP, Caldeo V, Kelly AL, O'Mahony JA. 2016. Impact of α-lactalbumin: β-lactoglobulin ratio on the heat stability of model infant milk formula protein systems. Food Chemistry. 194: 184–190. https://doi.org/10.1016/j.foodchem.2015.07.141

Dalgleish DG. 1990. The effect of denaturation of β-lactoglobulin on renneting: a quantitative study. Milchwissenschaft. 45: 491–494.

Dario C, Carnicella D, Dario M, Bufano G. 2008. Genetic polymorphism of β-lactoglobulin gene and effect on milk composition in Leccese sheep. Small Ruminant Research. 74: 270–273. https://doi.org/10.1016/j.smallrumres.2007.08.003

De Pascale S, Caira S, Garro G, Mauriello R, Scaloni A, Cosenza G, Chianese L. 2022. Proteomic characterisation and phylogenetic derivation of ovine α_{S1}-CN B and α_{S1}-CN G genetic variants. International Dairy Journal. 131:105387.

El-Zahar K, Sitohy M, Dalgalarrondo M, Choiset Y, Métro F, Haertlé T, Chobert JM. 2004. Purification and physicochemical characterization of ovine β-lactoglobulin and α-lactalbumin. Food/Nahrung. 48:177-183.

Fang ZH, Visker M, Miranda G, Delacroix-Buchet A, Bovenhuis H, Martin P. 2016. The relationships among bovine αS-casein phosphorylation isoforms suggest different phosphorylation pathways. Journal of Dairy Science. 99:8168-8177.

Farrell Jr H, Jimenez-Flores R, Bleck G, Brown E, Butler J, Creamer L, Hicks C, Hollar C, Ng-Kwai-Hang K, Swaisgood H. 2004. Nomenclature of the proteins of cows' milk—Sixth revision. Journal of Dairy Science. 87:1641-1674.

Ferranti P, Malorni A, Nitti G, Laezza P, Pizzano R, Chianese L, Addeo F. 1995. Primary structure of ovine αsl-caseins: localization of phosphorylation sites and characterization of genetic variants A, C and D. Journal of Dairy Research. 62:281-296.

Ford GD, Grandison AS. 1986. Effect of size of casein micelles on coagulation properties of skim milk. Journal of Dairy Research. 53:129-133.

Frederiksen P, Andersen K, Hammershøj M, Poulsen H, Sørensen J, Bakman M, Qvist K, Larsen L. 2011. Composition and effect of blending of noncoagulating, poorly coagulating, and well-coagulating bovine milk from individual Danish Holstein cows. Journal of Dairy Science. 94:4787-4799.

Gai N, Uniacke-Lowe T, O'Regan J, Faulkner H, Kelly AL. 2021. Effect of protein genotypes on physicochemical properties and protein functionality of bovine milk: a review. Foods. 10:2409.

Garcia-Gamez E, Gutierrez-Gil B, Sahana G, Sanchez JP, Bayon Y, Arranz JJ. 2012. GWA analysis for milk production traits in dairy sheep and genetic support for a QTN influencing milk protein percentage in the LALBA gene. Plos One 7: e47782.

Garzon AI, Martinez J. 1992. β-lactoglobulin in Manchega sheep breed: Relationship with milk technological index in handcraft manufacture of manchego cheese. International Conference on Animal Genetetics. 1992.

Giambra I, Brandt H, Erhardt G. 2014. Milk protein variants are highly associated with milk performance traits in East Friesian Dairy and Lacaune sheep. Small Ruminant Research. 121:382-394.

Huppertz T, Fox PF, Kelly AL. 2018. The caseins: Structure, stability, and functionality. In: Proteins in food processing. Woodhead Publishing. p. 49-92.

Ibrahim WI, Hassooni HA, Alkhazraji WJ. 2019. Association of β-lactoglobulin gene polymorphism with milk production and composition in local Awassi sheep. Plant Archives. 19:284-288.

Imafidon GI, Ng-Kwai-Hang K, Harwalkar V, Ma CY. 1991. Effect of genetic polymorphism on the thermal stability of β-lactoglobulin and κ-casein mixture. Journal of Dairy Science. 74:1791-1802.

Jakob E, Puhan Z. 1992. Technological properties of milk as influenced by genetic polymorphism of milk proteins—A review. International Dairy Journal. 2:157-178.

Jõudu I, Henno M, Kaart T, Püssa T, Kärt O. 2008. The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. International Dairy Journal. 18:964-967.

Kawęcka A, Radkowska I. 2023. Comparison of the quality of mountain sheep milk obtained from animals kept on a natural and organic mountain pasture. Annals of Animal Science. 23:275-283.

Keppler JK, Sönnichsen FD, Lorenzen PC, Schwarz K. 2014. Differences in heat stability and ligand binding among β-lactoglobulin genetic variants A, B and C using 1H NMR and fluorescence quenching. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 1844:1083-1093.

Ketto IA, Knutsen TM, Øyaas J, Heringstad B, Ådnøy T, Devold TG, Skeie SB. 2017. Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. International Dairy Journal. 70:55-64.

Ketto IA, Abdelghani A, Johansen AG, Skeie SB, Øyaas J. 2019. Effect of milk protein genetic polymorphisms on rennet and acid coagulation properties after standardisation of protein content. International Dairy Journal. 88:18-24.

Li S, Ye A, Singh H. 2019. Seasonal variations in composition, properties, and heat-induced changes in bovine milk in a seasonal calving system. Journal of Dairy Science. 102:7747-7759.

Marshall AC, Lopez-Villalobos N, Loveday SM, Ellis A, McNabb W. 2023. Modelling Lactation Curves for Dairy Sheep in a New Zealand Flock. Animals. 13:349.

Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024. Animal factors affecting the cheese-making properties and the heat coagulation time of milk from dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. 1-18.

Martin P, Cebo C, Miranda G. 2013. Interspecies comparison of milk proteins: quantitative variability and molecular diversity. In: Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects. 4th Edition. p. 387-429.

Marziali A, Ng-Kwai-Hang K. 1986. Effects of milk composition and genetic polymorphism on coagulation properties of milk. Journal of Dairy Science. 69:1793-1798.

McLean DM, Graham ERB, Ponzoni RW. 1987. Effects of milk protein genetic variants and composition on heat stability of milk. Journal of Dairy Research. 54:219-235.

McMahon DJ, Brown RJ. 1982. Evaluation of Formagraph for comparing rennet solutions. Journal of Dairy Science. 65:1639-1642.

Moatsou G, Samolada M, Katsabeki A, Anifantakis E. 2004. Casein fraction of ovine milk from indigenous Greek breeds. Le Lait. 84:285-296.

Moioli B, Pilla F, Tripaldi C. 1998. Detection of milk protein genetic polymorphisms in order to improve dairy traits in sheep and goats: a review. Small Ruminant Research. 27:185-195.

Noce A, Pazzola M, Dettori ML, Amills M, Castelló A, Cecchinato A, Bittante G, Vacca GM. 2016. Variations at regulatory regions of the milk protein genes are associated with milk traits and coagulation properties in the Sarda sheep. Animal Genetics. 47:717-726.

Nudda A, Feligini M, Battacone G, Macciotta NPP, Pulina G. 2003. Effects of lactation stage, parity, β-lactoglobulin genotype and milk SCC on whey protein composition in Sarda dairy ewes. Italian Journal of Animal Science. 2:29-39.

Othmane MH, Carriedo JA, De la Fuente Crespo LF, San Primitivo F. 2002. An individual laboratory cheese-making method for selection in dairy ewes. Small Ruminant Research. 45:67-73.

O'Connell J, Fox P. 2001. Effect of beta-lactoglobulin and precipitation of calcium phosphate on the thermal coagulation of milk. The Journal of Dairy Research. 68:81-94.

Pelmus RS, C PG, Lazar C, Marin DE, Gras M, Radu M, Ghita E. 2012. Preliminary study on milk composition and milk protein polymorphism in the Romanian local sheep breed Teleorman Black Head Tsigai. Romanian Biotechnological Letters. 17:7583.

Picariello G, Rignanese D, Chessa S, Ceriotti G, Trani A, Caroli A, Di Luccia A. 2009. Characterization and genetic study of the ovine α_{s2}-casein (CSN1S2) allele B. The Protein Journal. 28:333–340.

Picariello G, Di Luccia A, Ferranti P, Alloggio I, Addeo F, Pieragostini E. 2012. A proteomic approach to investigate the qualitative and quantitative polymorphism of β-lactoglobulin in ovine milk: Inference on gene copy-number variations. Advances in Biological Chemistry. 2012.

Piredda G, Papoff CM, Sanna SR, Campus RL. 1993. Influenza del genotipo della α_{s1}-caseina ovina sulle caratteristiche chimico-fisiche e lattodinamografiche del latte. Sci. Tecn. Latt.-Cas. 44:135–143.

Pirisi A, Fraghi A, Piredda G, Leone P. 1999a. Influence of sheep AA, AB and BB β-lactoglobulin genotypes on milk composition and cheese yield. Proceedings of the Sixth International Symposium on the Milking of Small Ruminants, Athens, Greece.

Pirisi A, Piredda G, Papoff CM, Di Salvo R, Pintus S, Garro G, Ferranti P, Chianese L. 1999b. Effects of sheep α_{s1}-casein CC, CD and DD genotypes on milk composition and cheesemaking properties. Journal of Dairy Research. 66:409–419.

Ramos A, Matos C, Russo-Almeida P, Bettencourt C, Matos J, Martins A, Pinheiro C, Rangel-Figueiredo T. 2009. Candidate genes for milk production traits in Portuguese dairy sheep. Small Ruminant Research. 82:117–121.

Richardson BC, Mercier JC. 1979. The primary structure of the ovine β-caseins. European Journal of Biochemistry. 99:285–298.

Sacchi P, Chessa S, Budelli E, Bolla P, Ceriotti G, Soglia D, Rasero R, Cauvin E, Caroli A. 2005. Casein haplotype structure in five Italian goat breeds. Journal of Dairy Science. 88:1561–1568.

Sallam AM. 2023. Effect of genetic polymorphisms in LALBA and Prolactin genes on milk traits in Barki ewes. Small Ruminant Research. 226:107041.

Schmidt D. 1970. Differences between the association of the genetic variants B, C and D of α_{s1}-casein. Biochimica et Biophysica Acta. 221:140–142.

Schmidt D, Ebner K. 1972. Multiple forms of pig, sheep and goat α-lactalbumin. Biochimica et Biophysica Acta (BBA) - Protein Structure. 263:714–720.

Selvaggi M, Laudadio V, Dario C, Tufarelli V. 2014. Investigating the genetic polymorphism of sheep milk proteins: a useful tool for dairy production. Journal of the Science of Food and Agriculture. 94:3090–3099.

Stacey A, Schnieke A, Kerr M, Scott A, McKee C, Cottingham I, Binas B, Wilde C, Colman A. 1995. Lactation is disrupted by alpha-lactalbumin deficiency and can be restored by human alpha-lactalbumin gene replacement in mice. Proceedings of the National Academy of Sciences. 92:2835–2839.

Suárez-Vega A, Gutiérrez-Gil B, Klopp C, Tosser-Klopp G, Arranz JJ. 2017. Variant discovery in the sheep milk transcriptome using RNA sequencing. BMC Genomics. 18:1–13.

Swaisgood HE. 2003. Chemistry of the caseins. In: Advanced Dairy Chemistry—1 Proteins: Part A/Part B. Springer: 139–201.

Triantaphyllopoulos KA, Koutsouli P, Kandris A, Papachristou D, Markopoulou KE, Mataragka A, Massouras T, Bizelis I. 2017. Effect of β-lactoglobulin gene polymorphism, lactation stage and breed on milk traits in Chios and Karagouniko sheep breeds. Annals of Animal Science. 17:371–384.

Trujillo AJ, Casals I, Guamis B. 2000. Analysis of major ovine milk proteins by reversed-phase high-performance liquid chromatography and flow injection analysis with electrospray ionization mass spectrometry. Journal of Chromatography A. 870:371–380.

Vigolo V, Franzoi M, Cendron F, Salvadore G, Penasa M, Cassandro M, De Marchi M. 2022. Characterization of the genetic polymorphism linked to the β-casein A1/A2 alleles using different molecular and biochemical methods. Journal of Dairy Science. 105:8946–8955.

Vigolo V, Niero G, Penasa M, De Marchi M. 2022. Effects of preservative, storage time, and temperature of analysis on detailed milk protein composition determined by reversed-phase high-performance liquid chromatography. Journal of Dairy Science. 105:7917–7925.

Wedholm A. 2008. Variation in milk protein composition and its importance for the quality of cheese milk. Department of Food Science, Swedish University of Agricultural Sciences.

Yousefi S, Azari MA, Zerehdaran S, Samiee R, Khataminejhad R. 2013. Effect of β-lactoglobulin and κ-casein genes polymorphism on milk composition in indigenous Zel sheep. Archives Animal Breeding. 56:216–224.

Zhang W, Zheng S, Gao P, Ren Q, Zhang Y, Chen B, Hettinga K, Pang X, Lv J, Zhang S. 2023. Identification of the coagulation properties of Chinese Holstein bovine milk: Effects of milk compositions, milk protein polymorphism, and phosphorylation levels on milk coagulation ability. Food Hydrocolloids. 145:109112.

Appendix 2 (Supplementary information for Chapter 5)

Appendix 2 Table 1. Number of ewes with 2, 3, and ≥ 4 test-day records during the 2021-2022 milk production season.

Total records	Number of ewes
2 records	22
3 records	78
≥ 4 records	47
Total:	147

Appendix 2 Table 2. Frequencies of milk protein polymorphisms, as aggregate casein proteins (α_{s1}/ α_{s2}/β-CN) and as separate whey protein fractions, in a New Zealand dairy sheep flock, before removal of least frequent (< 7%) polymorphisms.

Protein[1]	Polymorphism[2]	Number of ewes	Frequency in the population (%)
Casein	AA/AA/AA	101	61
(α_{s1}/ α_{s2}/β-CN)	AB/AB/AB	52	32
	other combinations	12	7
	Total	165	
Whey fractions			
α-LA	AA	51	31
	AB	75	45
	BB	39	24
	Total	165	
β-LG	AA	118	72
	AB	43	26
	BB	4	2
	Total	165	

[1] α_{s1}/ α_{s2}/β-CN= aggregate (haplotype) of α_{s1}-casein, α_{s2}-casein, and β-casein proteins; α-LA= α-lactalbumin; β-LG= β-lactoglobulin.

[2] Nomenclature was self-given to the protein polymorphisms in this population of dairy sheep, as they were being identified in the RP-HPLC chromatogram. Genomic studies and other laboratorial techniques for the description of protein structure will enable the confirmation of any protein genetic variants previously reported in the literature.

Appendix 2 Table 3. F-value for effects of animal factors on milk yield and composition, contents of calcium and urea, SCS, protein composition, milk coagulation properties, cheese yield, HCT, and pH for dairy sheep milked once-a-day during the 2021-2022 production season.

Trait[1]	Coat colour	Litter size	Age	Stage of lactation	dmld[2]
Milk yield (L/day)	0.55	0.03	6.20***	167.70***	3.13
Fat (%)	0.52	1.25	0.18	168.13***	1.74
Protein (%)	0.33	4.54*	4.04**	482.15***	0.03
Lactose (%)	0.14	0.01	0.80	327.98***	0.01
Casein (%)	0.62	3.40	3.31*	400.82***	0.08
Casein:Protein	27.37***	1.18	1.49	3.76*	2.71
Calcium (mg/100 mL)	1.54	0.01	3.22*	49.86***	3.71
Urea (mg/100 mL)	3.93*	0.04	0.23	71.98***	4.94*
SCS (Log_2SCC)	1.79	1.97	1.56	18.23***	1.73
Protein composition (% TP)					
κ-casein	13.96***	0.27	3.81**	90.97***	26.34***
α_{s1}-casein	9.5**	0.03	1.38	52.63***	8.12**
α_{s2}-casein	12.22***	0.69	3.71**	14.70***	1.98
β-casein	3.22	0.10	4.41**	9.07***	0.38
α-lactalbumin	8.87**	1.34	1.98	172.66***	0.25
β-lactoglobulin	4.91*	0.14	0.11	67.24***	1.67
Milk coagulation properties					
RCT (min)	3.06	7.61**	0.62	29.29***	0.71
K20 (min)	0.09	7.48**	3.53*	27.48***	0.39
A30 (mm)	0.06	8.83**	2.83*	27.94***	0.43
ILCY (%)	7.19**	0.32	6.00***	17.02***	0.01
HCT (min)	8.57**	0.24	2.21	18.28***	2.83
pH	3.6	0.12	7.28***	141***	2.76

[1] Casein:Protein= ratio of casein content to protein content, calculated as casein (%) divided by protein (%); SCS=somatic cell score (Log_2 SCC); SCC= somatic cell count; TP= total protein; RCT= rennet coagulation time; K_{20}= time to reach curd firmness of 20 mm; A_{30}= curd firmness at 30 min post rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time. [2]dmld= deviation from the median lambing date of the flock. Statistical significance is given as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Appendix 2 Table 4. Least squares means (and standard errors) of protein composition (% in total protein) for different classes of ewe colour, litter size, age (parity), and for different stages of lactation.

		Content of protein fraction (% in total protein)[2]					
Effect		κ-CN	α_{s1}-CN	α_{s2}-CN	β-CN	α-LA	β-LG
Coat colour	Black	$8.12^{a} \pm 0.17$	$26.40^{b} \pm 0.33$	$13.78^{a} \pm 0.30$	39.48 ± 0.30	$2.23^{a} \pm 0.01$	$10.00^{b} \pm 0.19$
	White	$7.14^{b} \pm 0.07$	$27.47^{a} \pm 0.14$	$12.66^{b} \pm 0.13$	40.06 ± 0.13	$1.93^{b} \pm 0.04$	$10.45^{a} \pm 0.08$
Litter size	1 lamb	7.73 ± 0.12	26.95 ± 0.23	13.12 ± 0.21	39.80 ± 0.22	2.13 ± 0.06	10.25 ± 0.13
	≥2 lambs	7.80 ± 0.11	26.91 ± 0.21	13.32 ± 0.20	39.73 ± 0.20	2.04 ± 0.06	10.19 ± 0.12
Age	1 year-old	$8.87^{a} \pm 0.25$	26.64 ± 0.47	$13.74^{a} \pm 0.43$	$39.17^{b} \pm 0.44$	1.96 ± 0.13	10.13 ± 0.27
	2 years-old	$7.71^{b} \pm 0.14$	26.85 ± 0.26	$13.48^{a} \pm 0.24$	$39.40^{b} \pm 0.24$	2.22 ± 0.07	10.28 ± 0.15
	3 years-old	$7.46^{b} \pm 0.14$	26.88 ± 0.25	$13.01^{ac} \pm 0.23$	$40.25^{a} \pm 0.24$	2.11 ± 0.07	10.26 ± 0.15
	≥4 years-old	$7.52^{b} \pm 0.12$	27.35 ± 0.23	$12.64^{bc} \pm 0.21$	$40.24^{a} \pm 0.21$	2.03 ± 0.06	10.21 ± 0.13
Stage of lactation[1]	1 (≥50 and <95 days)	$8.79^{a} \pm 0.14$	$26.12^{c} \pm 0.21$	$13.42^{a} \pm 0.19$	$39.25^{b} \pm 0.22$	$2.44^{a} \pm 0.06$	$9.97^{b} \pm 0.13$
	2 (≥95 and <140 days)	$7.65^{b} \pm 0.11$	$26.98^{b} \pm 0.19$	$13.38^{a} \pm 0.17$	$39.97^{a} \pm 0.18$	$2.22^{b} \pm 0.57$	$9.80^{b} \pm 0.11$
	3 (≥140 and ≤182 days)	$6.87^{c} \pm 0.12$	$27.69^{a} \pm 0.20$	$12.86^{b} \pm 0.18$	$40.08^{a} \pm 0.20$	$1.59^{c} \pm 0.06$	$10.90^{a} \pm 0.12$

[1] Stage of lactation: categorical variable representing three different ranges of days in milk (date of test − date of lambing).

[2] κ-CN= κ-casein; α_{s1}-CN= α_{s1}-casein; α_{s2}-CN= α_{s2}-casein; β-CN= β-casein; α-LA= α-lactalbumin; β-LG= β-lactoglobulin.

[a, b, c] Least squares means with different superscripts, within effect, are significantly different ($p < 0.05$).

Chapter 6

Estimation of genetic parameters for production, composition, and processability of milk from dairy sheep in a New Zealand flock

This chapter has been published elsewhere. It has been reformatted and presented here with permission:

Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024. Estimation of genetic parameters for production, composition and processability of milk from dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. Published online: 27 Jun 2024. https://doi.org/10.1080/00288233.2024.2368505

Abstract

This paper aimed to estimate the heritability and genetic correlations for processability (milk coagulation properties, cheese yield, and heat stability), milk production, and milk composition of dairy sheep in a New Zealand flock. Test-day records from 169 ewes were obtained during the 2021-2022 season. Heritability estimates for yield traits (milk, fat, protein, and lactose yield) were moderate and ranged from 0.33 to 0.38. Whereas heritability estimates for processability traits were lower and ranged from 0.17 to 0.27. The genetic correlations indicated that poorer milk coagulation properties were associated with lower mammary health indicated by higher SCS, higher pH, and lower lactose content. Additionally, a higher ratio of casein to calcium i.e. lower availability of soluble calcium was associated with poorer milk coagulation properties. Higher yields of milk, fat, protein, and lactose were associated, but to a smaller degree, with better MCP. It is proposed that genetic improvement of dairy sheep for higher yields of fat and protein, and for lower SCS, could indirectly improve milk processability for cheese-making. However, it is recommended that larger studies involving more dairy sheep flocks are conducted to validate the present results before developing a selection index for this industry.

Keywords

Milk coagulation properties; Milk processability; Milk Production; Genetic parameters; Dairy sheep.

6.1 Introduction

The New Zealand sheep dairy industry is developing and complementing the existing cow dairy industry, with estimates to generate $750 M through exports by 2035 (Scale up 2022). Exports currently mainly consist of milk powder and infant formula. This sector is also diversifying the domestic market with alternative products to consumers looking for specific health benefits or experiencing intolerance to milk from other species. Additionally, restaurants and enthusiasts of gourmet food seek sheep milk products, particularly cheeses (Lees and Lees 2016). Furthermore, dairy sheep farming has the potential to have a lower environmental impact (Downie-Melrose 2014), but this is still under further investigation (McCoard 2023). Moreover, the pay for sheep milk is appealing to dairy farmers as it reached between $16 to $17 per kilogram of milk solids in 2021 (Gullery 2021).

However, in general, dairy sheep in New Zealand have been considered low producing when compared to dairy sheep in Europe, which has been attributed to a slow rate of genetic progress and suboptimal feed management practices for pasture-based systems (Lees and Lees 2016). There has been noted a large gap between low (60 to 90 litres of milk in a season) and high producing dairy ewes (300 to 400 litres a year) in New Zealand flocks, evidencing the potential of genetic improvement (Gullery 2021; MPI and Massey University 2020). The primary genetic makeup of New Zealand dairy sheep comprises East-Friesian breed (Peterson and Prichard 2015) alongside some genetic lines of meat breeds such as Coopworth and Border Leicester. Additionally, some flocks have more recently incorporated other breeds such as the Awassi and Lacaune, introduced by the major companies (New Zealand Sheepbreeders Association 2024 a,b,c).

Genetic improvement of livestock is one of the ways of increasing animal production efficiently and sustainably, with due consideration for animal health and welfare (Flint and Woolliams 2008). However, the significant costs associated with certified milk meters, data collection, pedigree management, and expertise in statistical genetic analyses have restricted advanced breeding programs to large dairy sheep companies. Nonetheless, implementing selection programs in smaller flocks can yield positive economic and social impacts, preserve breeds, and facilitate genetic progress even in small animal populations.

Estimating genetic parameters, such as heritability and genetic correlations, is crucial for developing selection indexes and predicting response to selection (Lopez-Villalobos 2012), as selection of one trait can result in undesirable side effects (Flint and Woolliams 2008). Therefore, it is important to consider the impact that the selection of dairy sheep on production traits solely would have on milk processability, since sheep milk is mainly used for processing into dairy products, rather than being consumed as fresh milk (Bencini and Pulina 1997). The genetic bases of processability traits have yet to be explored in dairy sheep in New Zealand.

Considering this context, the objective of this study was to estimate heritability, genetic and phenotypic correlations of milk processability traits, milk production, and milk composition in dairy sheep from a New Zealand flock. These preliminary estimations of genetic parameters will provide insights for the creation of a selection index to rank ewes and rams in dairy sheep flocks dedicated to cheese production and highlight the importance of advancing genetic research within the dairy sheep industry on a wider scale.

6.2 Materials and methods

An animal ethics approval was obtained for this study (MUAEC Protocol 21/45). Data were collected from a commercial flock of 169 ewes in Masterton, New Zealand. Description of the animals and of the farm has been previously reported (Marshall et al. 2023). The development of the breed started 27 years ago using East-Friesian genetics, and some Coopworth and Border Leicester genetics.

The farm operates on an extensive seasonal grazing system; the ewes graze clover and lucerne pasture and have limited access to supplementary feed during milking. Mating with rams started in mid-March. Lambs were born between July and November, with median lambing date of 20th August 2021. The farm has an exclusive suckling period, so milking for artisan cheese production starts after the lambs are fully weaned at the discretion of the farmer. In the 2021–2022 season, the average suckling period was 57 days. Milk collection for cheese production happened from early October to mid-February. Milking frequency was twice-a-day during the entire month of October and was shifted to once-a-day on the 1st of November, this is a common practice at the farm to align milk production with pasture availability.

Masterton has hot and dry summers and wet winters with some frosts. Maximum temperature was 30.2°C, and minimum temperature was -0.2°C during the milk production season. January was the hottest month with the lowest rainfall (13.4 mm) (Meteorological Service NZ Ltd 2022). Pasture quality varied significantly. In October 2021 pasture contained 12.0 MJ of metabolizable energy/kg DM and 26.7 % of crude protein (% DM). The level of metabolizable energy and crude protein in the pasture dropped 32% and 47%, respectively in January 2022, whereas the level of non-detergent-fibre increased 40%, resulting in a decrease of 31% in organic dry matter digestibility. More detailed information on pasture nutritional value is provided by Marshall et al. (2023, Chapter 3).

6.2.1 Test-day records

A total of 639 test-day records from 169 dairy sheep were gathered from the start of the milking season (04 October 2021) until 31 January 2022, for information on milk production, composition, and processability. At least two milk tests were obtained from

each ewe from 35 to 182 days in milk, 78% of the flock had a minimum of four records, 17% had a minimum of three records, and 5% had two records throughout the season.

6.2.2 Milk production

The milk yield from individual ewes was manually recorded as the total volume taken from individual test buckets attached to the cups and to the milk line, and a representative milk sample was taken from the total milk produced by each ewe for compositional and processability analyses. The milk samples were immediately stored in a cooler with ice during collection for transportation within four hours to Palmerston North.

For the statistical analysis of the data from twice-a-day milking, the volume from the afternoon milking and the volume of the following morning milking were added to obtain the test-day milk yield. The corresponding composition traits for that day were the weighted afternoon and morning milk volumes. There was no statistical difference in MCP between afternoon and morning samples, therefore MCPs were averages of afternoon and morning samples. Once-a-day milking happened in the afternoons.

Information on pedigree, litter size, and parity number of ewes were available for this study.

6.2.3 Milk composition

Sodium azide was added to milk samples (to final concentration of 0.025% w/w) on arrival in Palmerston North, prior to aliquoting for the different analyses. An aliquot was analysed by Milk Test NZ Ltd (Hamilton, NZ) using a Combi FOSS instrument (Foss Analytics). The composition analysis included percentages of fat, protein, casein, lactose, and SCC (cells/mL) converted into somatic cell scores (SCS) using a Log_2 transformation. The analyses for percentage of casein and urea (mg/100mL) were performed using a Fourier-transform Infrared (FTIR) milk-analyser MilkoScan FT6000 (Foss Analytics) calibrated for sheep milk samples. Milk samples were also submitted at a contract laboratory (Massey Nutrition Lab) for analysis of total calcium content (mg/100 mL). The ratio of casein to protein was calculated as casein percentage divided by protein percentage, multiplied by 100. The same rule applied for calculation of ratio of casein to fat, and ratio of casein to calcium content.

6.2.4 Milk processability

Milk coagulation properties

Traditional milk coagulation properties (MCP) were assessed using a Formagraph instrument (Foss Analytics). For each individual milk sample, 10 mL was warmed to 31°C before adding the 200 μL of the rennet solution to achieve 0.0513 IMCU/mL of milk (Cipolat-Gotet et al. 2016). The rennet solution was prepared with 0.24 g of commercial calf rennet powder 1100 IMCU/g (92% chymosin and 8% pepsin) diluted in 100 mL of pure water.

After rennet addition, Formagraph analysis was conducted for 30 minutes at 31°C. Curd firmness (measured in millimeters) was recorded at 0.125-minute intervals (7.5 seconds). The traditional MCP extracted from the Formagraph indicate the quality of milk for cheesemaking, these included rennet coagulation time (RCT, min), curd firmness at 30 minutes (A30, mm), and time to reach curd firmness of 20 mm (K20, min) (McMahon and Brown 1982).

Milk pH was measured at 31°C, using a calibrated pH meter (EcoScan Model pH5) on the same day as milk coagulation properties were obtained.

Individual laboratory cheese yield

Due to practical constraints, a subset of 372 samples and a minimum of two records per ewe, taken during once-a-day milking period, were used for manufacturing of fresh soft curds to measure individual laboratory cheese yield (ILCY), following protocols from Othmane et al. (2002) and Manca et al. (2016), with temperature adjustment to 31°C, as described in Marshall et al. (2024), Chapter 4.

Heat stability

A total of 344 milk samples and a minimum of two records per ewe, taken during once-a-day milking period, were used to measure heat coagulation time (HCT). Whole milk in glass tubes were immersed and oscillated in an oil bath set at 140°C, also described in Marshall et al. (2024), Chapter 4. The time at which coagulation particles became visible was recorded.

6.2.5 Statistical analyses

Descriptive statistics (mean, standard deviation, minimum and maximum values) for MCPs, milk production and composition traits, SCS and pH were obtained using the MEAN procedure in SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA).

Estimates of (co)variance components for animal additive genetic variance (σ^2_a), permanent environment variance (σ^2_c), and residual (σ^2_e) were obtained by fitting univariate and bivariate repeatability animal models as implemented in the JWAS package (Cheng et al. 2018) of Julia version 1.7.3, using Bayesian estimation method and Markov chain Monte Carlo procedures. Marginal posterior distributions of all unknowns were estimated using the Gibbs sampling algorithm. Inference was based on 1,000 samples retained from chain lengths of 100,000 samples and burning of 10,000. The following repeatability model was used:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wc} + \mathbf{e}$$

where **y** is the vector of records for traits 1 and 2; **X** is the design matrix of fixed effects, **b** is the vector of fixed effects which included ewe coat colour as an indicator of variety within the breed (categorical variable with two levels: black or white), litter size (categorical variable with two levels: 1 lamb or 2 lambs and greater), ewe age (categorical variable with four levels: 1, 2, 3, and 4 years and older), milking frequency as a covariate (for records collected at twice- or at once-a-day milking, 1=once-a-day; 2=twice-a-day), and stage of lactation also as a covariate (time = days in milk), **Z** is the design matrix of random animal additive genetic effects (**u**), **W** is the design matrix of ewe permanent environment effects (**c**), and **e** is the vector of random residual errors.). The relationship matrix included 273 individuals, being 23 sires, 113 dams, and 103 founders.

Heritability (h^2) and repeatability (t) for traits were calculated using the estimated variance components. The heritability of a trait was calculated as the proportion between the genetic variance and the phenotypic variance. The phenotypic variance σ^2p was calculated as the sum of animal genetic variance, permanent environment, and residual (σ^2a, σ^2c, and σ^2e). The repeatability of the trait was calculated as the proportion of permanent variance (the sum of σ^2a and σ^2c) and the phenotypic variance.

$$h^2 = \frac{\sigma^2_a}{\sigma^2_p}$$

$$\sigma_p^2 = \sigma_a^2 + \sigma_c^2 + \sigma_e^2$$

$$t = \frac{\sigma_a^2+\sigma_c^2}{\sigma_p^2}$$

Genetic (r_G) and phenotypic (r_P) correlations are calculated as follows (Falconer and Mackay 1996):

$$r_G = \frac{\sigma_{a1a2}}{\sigma_{a1} \times \sigma_{a2}} \text{ and } r_P = \frac{\sigma_{p1p2}}{\sigma_{p1} \times \sigma_{p2}},$$

where σ_{a1a2} is the animal (genotypic) covariance between traits 1 and 2; σ_{p1p2} is the phenotypic covariance between traits 1 and 2; σ_{a1} and σ_{p1} are the additive genetic and phenotypic standard deviations for trait 1, respectively; and σ_{a2} and σ_{p2} are the additive genetic and phenotypic standard deviations for trait 2.

The 95% credibility intervals were computed by setting the 97.5th percentile of the MCMC samples as the upper limit and the 2.5th percentile as the lower limit.

6.3 Results

The means, standard deviations, minimum and maximum values for each dependent variable are shown in Table 6.1. The studied flock of dairy sheep produced an average of 660 mL of milk per day per ewe from 35 to 182 days during the 2021-2022 milk production season, which included a transition of milking frequency from twice-a-day to once-a-day. Milk contained an average of 6.12% fat and 5.39% protein.

Table 6.1. Means, standard deviations (SD), minimum and maximum values for milk production, composition, and milk processability traits for dairy sheep milked in mid and late lactation during the 2021-2022 season.

Trait[1]	N	Mean	SD	Minimum	Maximum
MY (mL/day)	639	660	350	100	1900
FY (g/day)	639	38.95	18.29	3.82	116.44
PY (g/day)	639	34.62	16.09	4.77	98.37
LY (g/day)	639	32.13	17.74	3.76	95.69
CSNY (g/day)	584	26.29	12.08	3.76	76

FP (%)	639	6.12	1.27	1.87	11.24
PP (%)	639	5.39	0.7	3.78	8.8
LP (%)	639	4.76	0.27	3.66	5.54
CSNP (%)	584	4.14	0.5	2.87	6.28
CSNP:PP	584	76	2	54	92
CSNP:FP	584	70	15	44	191
CSNP:Calcium	584	22	3	15	40
Calcium (mg/100 mL)	630	188.0	25.9	104.6	269.4
Urea (mg/ 100 mL)	584	41.7	9.01	9.39	71.28
SCS (Log_2SCC)	639	16.27	2.02	9.97	24.58
RCT (minutes)	603	14.46	5.97	6.86	26.5
K20 (minutes)	596	3.56	3.24	1.3	10.2
A30 (millimetres)	603	49.88	15.39	7.4	79.2
ILCY (%)	372	44.7	9.4	23.4	89.6
HCT (min)	344	1.43	0.83	0.07	5.00
pH	639	6.61	0.21	6.30	7.14

[1] MY= milk yield; FY= fat yield; PY= protein yield; LY= lactose yield; CSNY= casein yield; FP= fat percentage; PP= protein percentage; LP= lactose percentage; CSNP= casein percentage; CSNP:PP= ratio of casein to protein; CSNP:FP=ratio of casein to fat; CSNP:Calcium= ratio of casein to calcium; SCS=somatic cell score; RCT= rennet coagulation time after rennet addition; K20= time to reach curd firmness of 20 mm after coagulation; A30= curd firmness at 30 min after rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time.

During the same period, the milk samples of the individual ewes took, on average, 14.46 min to start coagulating after rennet addition, 3.56 min to reach curd firmness of 20 mm after coagulation was initiated, and formed a curd of 49.88 mm of firmness at 30 minutes of analysis. From the total of 639 samples, 36 samples (5.6% of the samples) did not coagulate within 30 minutes of rennet addition, and 43 samples (6.7% of the samples) did not reach curd firmness of 20 mm in the Formagraph. These non-coagulating samples were given penalty values of 30 minutes for RCT, 15 minutes for K20 and 1 mm for A30 for the genetic analyses. Average ILCY produced by the flock was 44.7% and average HCT of was 1.43 minutes.

The estimated variances (additive genetic, permanent environment, and residual), heritabilities, and repeatabilities for each trait are presented in Table 6.2, for milk composition, and Table 6.3, for milk processability. Heritability estimates calculated in this study ranged from 0.12 (CSNP:FP) to 0.48 (PP), and repatability estimates ranged

from 0.21 (CSNP:FP) to 0.62 (PP). The 95% confidence intervals were large. The additive genetic variance had a large contribution to the total observed phenotypic variance for some traits, seen by the moderate heritability values obtained for yield traits, PP, LP, and CSNP, which ranged from 0.33 (FY) to 0.48 (PP). Heritability of processability traits were low to moderate and ranged from 0.17 (ILCY) to 0.27 (RCT).

The genetic and phenotypic correlations between milk production, composition, and processability traits are presented in Table 6.4. Strong absolute correlation values (r_G > |0.70|) were obtained amongst yield traits, between CSNP and PP, and amongst MCP. Yield traits were weakly correlated with composition traits ($-0.11 < r_G \leq 0.11$) and were weak to moderately correlated to MCP ($|0.10| < r_G \leq |0.25|$).

Moderate correlation values (r_G > |0.20|) were obtained between RCT and LY, PP, LP, CSNP:PP, CSNP:Calcium, and SCS. Stronger correlations (r_G > |0.40|) were obtained between milk pH and processability traits RCT, K20, A30, and HCT. ILCY was strongly correlated to composition traits FP, PP, CSNP, and calcium content (r_G > |0.40|), and HCT was moderately correlated to MCPs ($|0.20| < r_G \leq |0.40|$).

Table 6.2. Estimates of animal additive genetic (σ^2_a), ewe permanent environment (σ^2_c) and residual variances (σ^2_e), heritability (h^2) and repeatability (t), and their corresponding confidence intervals (CI), for milk production and composition, for dairy sheep milked in mid and late lactation during the 2021-2022 season.

Trait[1]	σ^2_a	95% CI	σ^2_c	95% CI	σ^2_e	95% CI	h^2	95% CI	t	95% CI
MY	199.3	(94.69 - 346.85)	129.86	(58.37 - 237.79)	301.74	(260.18 - 356.22)	0.38	(0.23 - 0.52)	0.52	(0.42 - 0.61)
FY	63.09	(26.27 - 124.71)	54.53	(21.41 - 96.89)	110.38	(94.32 - 129.03)	0.33	(0.18 - 0.50)	0.51	(0.42 - 0.61)
PY	51.40	(23.00 - 95.01)	33.53	(13.40 - 64.16)	75.88	(65.26 - 88.60)	0.38	(0.22 - 0.53)	0.53	(0.43 - 0.62)
LY	49.60	(25.26 - 83.33)	31.35	(13.54 - 59.76)	76.80	(66.21 - 88.34)	0.38	(0.24 - 0.5)	0.51	(0.42 - 0.60)
CSNY	26.14	(12.75 - 48.02)	21.68	(9.13 - 37.81)	40.27	(34.10 - 47.46)	0.37	(0.22 - 0.52)	0.54	(0.44 - 0.63)
FP	19.30	(10.07 - 32.31)	11.58	(5.38 - 21.21)	75.46	(65.35 - 87.41)	0.19	(0.11 - 0.27)	0.29	(0.20 - 0.38)
PP	10.50	(4.52 - 18.60)	5.89	(2.42 - 11.21)	10.08	(8.63 - 11.71)	0.48	(0.30 - 0.62)	0.62	(0.53 - 0.70)
LP	1.54	(0.70 - 2.65)	0.89	(0.35 - 1.77)	2.15	(1.86 - 2.51)	0.39	(0.23 - 0.52)	0.53	(0.44 - 0.62)
CSNP	5.59	(2.41 - 9.67)	3.14	(1.20 - 6.22)	5.84	(4.99 - 6.85)	0.46	(0.28 - 0.60)	0.60	(0.51 - 0.68)
CSNP:PP	1.14	(0.51 - 2.02)	0.62	(0.22 - 1.25)	2.47	(2.11 - 2.88)	0.30	(0.17 - 0.44)	0.41	(0.31 - 0.52)
CSNP:FP	27.38	(13.99 - 48.83)	17.8	(8.41 - 32.46)	174.48	(149.77 - 203.96)	0.12	(0.06 - 0.21)	0.21	(0.13 - 0.29)
CSNP:Calcium	1.82	(0.82 - 3.38)	1.11	(0.42 - 2.16)	5.44	(4.66 - 6.34)	0.23	(0.11 - 0.35)	0.35	(0.25 - 0.45)
Calcium	155.74	(68.99 - 282.34)	92.06	(36.43 - 181.20)	362.69	(314.1 - 422.09)	0.28	(0.16 - 0.41)	0.40	(0.31 - 0.51)
Urea	12.09	(5.94 - 21.39)	7.85	(3.48 - 15.21)	35.85	(30.48 - 41.94)	0.23	(0.12 - 0.35)	0.36	(0.26 - 0.46)
SCS	0.78	(0.35 - 1.48)	0.60	(0.21 - 1.16)	2.75	(2.35 - 3.19)	0.20	(0.10 - 0.32)	0.33	(0.23 - 0.44)

[1] MY= milk yield; FY= fat yield; PY= protein yield; LY= lactose yield; CSNY= casein yield; FP= fat percentage; PP= protein percentage; LP= lactose percentage; CSNP= casein percentage; CSNP:PP= ratio of casein to protein; CSNP:FP=ratio of casein to fat; CSNP:Calcium= ratio of casein to calcium; SCS=somatic cell score.

Table 6.3. Estimates of animal additive genetic (σ^2a), ewe permanent environment (σ^2c) and residual variances (σ^2e), heritability (h^2) and repeatability (t), and their corresponding confidence intervals (CI), for milk processability traits for dairy sheep milked in mid and late lactation during the 2021-2022 season.

Trait[1]	σ^2_a	95% CI	σ^2_c	95% CI	σ^2_e	95% CI	h^2	95% CI	t	95% CI
RCT	8.88	(3.36 - 16.37)	5.81	(2.03 - 11.47)	20.49	(17.59 - 23.83)	0.27	(0.12 - 0.41)	0.42	(0.31 - 0.52)
K20	1.94	(0.83 - 3.68)	1.28	(0.49 - 2.49)	7.13	(6.11 - 8.23)	0.19	(0.10 - 0.31)	0.31	(0.22 - 0.42)
A30	50.36	(19.95 - 98.02)	40.45	(15.33 - 76.43)	144.50	(124.15 - 168.58)	0.23	(0.11 - 0.38)	0.38	(0.28 - 0.49)
ILCY	13.71	(6.52 - 26.61)	8.23	(3.7 - 17.31)	62.22	(50.62 - 77.01)	0.17	(0.08 - 0.28)	0.26	(0.16 - 0.38)
HCT	0.13	(0.06 - 0.25)	0.07	(0.03 - 0.15)	0.53	(0.42 - 0.64)	0.18	(0.09 - 0.31)	0.27	(0.17 - 0.39)
pH	0.003	(0.001 - 0.005)	0.001	(0.001 - 0.002)	0.007	(0.006 - 0.008)	0.27	(0.16 - 0.39)	0.37	(0.28 - 0.47)

[1] RCT= rennet coagulation time after rennet addition; K20= time to reach curd firmness of 20 mm after coagulation; A30= curd firmness at 30 min after rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time.

Table 6.4. Genetic correlations (below diagonal) and phenotypic correlations (above diagonal) with confidence interval (in brackets) between milk production, composition, and processability traits, for dairy sheep milked in mid and late lactation during the 2021-2022 season.

Trait[1]	MY	FY	PY	LY	CSNY	FP	PP	LP	CSNP
MY		0.82	0.92	0.94	0.91	-0.09	-0.13	0.19	-0.10
		(0.77; 0.85)	(0.90; 0.94)	(0.92; 0.95)	(0.89; 0.94)	(-0.20; 0.02)	(-0.26; 0.00)	(0.06; 0.32)	(-0.24; 0.04)
FY	0.77		0.82	0.8	0.81	0.28	-0.05	0.11	-0.01
	(0.50; 0.91)		(0.78; 0.86)	(0.75; 0.84)	(0.77; 0.85)	(0.18; 0.38)	(-0.19; 0.09)	(-0.02; 0.24)	(-0.15; 0.13)
PY	0.86	0.78		0.91	0.95	-0.40	0.04	0.14	0.08
	(0.66; 0.93)	(0.49; 0.92)		(0.88; 0.93)	(0.93; 0.96)	(-0.15; 0.07)	(-0.10; 0.19)	(0.01; 0.27)	(-0.06; 0.21)
LY	0.88	0.76	0.84		0.90	-0.10	-0.14	0.27	-0.12
	(0.74; 0.95)	(0.48; 0.91)	(0.63; 0.93)		(0.87; 0.92)	(-0.20; 0.01)	(-0.27; -0.01)	(0.15; 0.38)	(-0.25; 0.02)
CSNY	0.82	0.71	0.87	0.82		-0.01	0.08	0.15	0.09
	(0.56; 0.93)	(0.34; 0.89)	(0.68; 0.96)	(0.57; 0.93)		(-0.12; 0.10)	(-0.06; 0.23)	(0.02; 0.28)	(-0.05; 0.23)
FP	-0.07	0.11	-0.01	-0.07	0.01		0.30	-0.20	0.28
	(-0.50; 0.34)	(-0.32; 0.50)	(-0.45; 0.43)	(-0.46; 0.32)	(-0.41; 0.47)		(0.19; 0.41)	(-0.30; -0.09)	(0.17; 0.39)
PP	-0.11	0.01	0.10	-0.14	0.09	0.35		-0.31	0.90
	(-0.51; 0.35)	(-0.46; 0.45)	(-0.38; 0.54)	(-0.52; 0.32)	(-0.46; 0.55)	(-0.09; 0.67)		(-0.42; -0.17)	(0.87; 0.92)
LP	0.10	0.08	0.02	0.20	0.10	-0.10	-0.35		-0.22
	(-0.36; 0.52)	(-0.41; 0.55)	(-0.44; 0.49)	(-0.25; 0.57)	(-0.38; 0.55)	(-0.50; 0.34)	(-0.70; 0.16)		(-0.34; -0.09)
CSNP	-0.12	-0.04	0.08	-0.20	0.08	0.34	0.87	-0.33	
	(-0.55; 0.32)	(-0.54; 0.50)	(-0.46; 0.51)	(-0.58; 0.25)	(-0.38; 0.52)	(-0.09; 0.66)	(0.67; 0.94)	(-0.65; 0.13)	
CSNP:PP	-0.09	-0.14	-0.19	-0.08	-0.12	-0.06	-0.24	0.08	-0.04
	(-0.51; 0.40)	(-0.59; 0.34)	(-0.61; 0.36)	(-0.53; 0.40)	(-0.57; 0.34)	(-0.50; 0.36)	(-0.60; 0.23)	(-0.39; 0.53)	(-0.47; 0.37)
CSNP:FP	-0.06	-0.16	-0.02	-0.11	-0.01	-0.42	0.32	-0.15	0.31
	(-0.48; 0.38)	(-0.62; 0.31)	(-0.45; 0.43)	(-0.52; 0.39)	(-0.47; 0.43)	(-0.38; -0.41)	(-0.14; 0.66)	(-0.54; 0.32)	(-0.22; 0.67)
CSNP:Ca	-0.10	-0.11	-0.04	-0.12	-0.04	-0.04	0.22	-0.23	0.21
	(-0.52; 0.36)	(-0.54; 0.36)	(-0.50; 0.44)	(-0.56; 0.32)	(-0.52; 0.45)	(-0.47; 0.40)	(-0.29; 0.65)	(-0.64; 0.34)	(-0.32; 0.58)

Table 6.4 (continuation). Genetic correlations with confidence interval (in brackets) between milk production, composition, and processability traits, for dairy sheep milked in mid and late lactation during the 2021-2022 season.

Trait[1]	MY	FY	PY	LY	CSNY	FP	PP	LP	CSNP
Ca	-0.04	0.06	0.06	-0.01	0.03	0.32	0.45	-0.04	0.54
	(-0.50; 0.41)	(-0.45; 0.54)	(-0.43; 0.52)	(-0.45; 0.41)	(-0.44; 0.50)	(-0.17; 0.65)	(-0.10; 0.74)	(-0.48; 0.45)	(0.14; 0.79)
Urea	0.19	0.01	0.12	0.19	0.11	-0.42	-0.30	0.05	-0.30
	(-0.25; 0.56)	(-0.49; 0.48)	(-0.34; 0.53)	(-0.24; 0.56)	(-0.37; 0.55)	(-0.71; 0.01)	(-0.64; 0.13)	(-0.39; 0.48)	(-0.65; 0.15)
SCS	-0.24	-0.28	-0.20	-0.27	-0.20	0.02	0.36	-0.47	0.22
	(-0.64; 0.25)	(-0.65; 0.21)	(-0.61; 0.34)	(-0.63; 0.21)	(-0.61; 0.32)	(-0.45; 0.49)	(-0.16; 0.72)	(-0.78; -0.02)	(-0.28; 0.65)
RCT	-0.19	-0.12	-0.12	-0.24	-0.03	0.02	0.34	-0.45	0.23
	(-0.61; 0.30)	(-0.62; 0.46)	(-0.57; 0.40)	(-0.62; 0.32)	(-0.11; 0.03)	(-0.45; 0.45)	(-0.18; 0.68)	(-0.75; 0.10)	(-0.32; 0.60)
K20	-0.12	-0.11	-0.13	-0.13	-0.04	-0.07	0.05	-0.33	-0.05
	(-0.55; 0.39)	(-0.55; 0.38)	(-0.56; 0.34)	(-0.54; 0.35)	(-0.04; -0.11)	(-0.50; 0.40)	(-0.45; 0.53)	(-0.69; 0.21)	(-0.52; 0.46)
A30	0.11	0.13	0.10	0.15	0.03	0.07	-0.08	0.34	0.03
	(-0.40; 0.55)	(-0.42; 0.58)	(-0.43; 0.54)	(-0.32; 0.58)	(-0.49; 0.55)	(-0.44; 0.49)	(-0.57; 0.41)	(-0.20; 0.72)	(-0.51; 0.54)
ILCY	-0.23	0.00	-0.12	-0.24	-0.13	0.58	0.43	-0.02	0.42
	(-0.63; 0.28)	(-0.47; 0.48)	(-0.58; 0.39)	(-0.62; 0.26)	(-0.58; 0.40)	(0.17; 0.82)	(-0.08; 0.77)	(-0.50; 0.47)	(-0.12; 0.76)
HCT	0.06	0.05	0.06	0.04	0.04	-0.07	-0.14	-0.22	-0.16
	(-0.45; 0.52)	(-0.53; 0.55)	(-0.48; 0.55)	(-0.45; 0.51)	(-0.48; 0.53)	(-0.51; 0.39)	(-0.59; 0.39)	(-0.64; 0.33)	(-0.63; 0.42)
pH	-0.18	-0.22	-0.18	-0.19	-0.19	-0.08	0.11	-0.24	0.06
	(-0.54; 0.27)	(-0.62; 0.23)	(-0.55; 0.25)	(-0.54; 0.27)	(-0.55; 0.23)	(-0.47; 0.32)	(-0.38; 0.52)	(-0.60; 0.22)	(-0.39; 0.48)

[1] MY= milk yield; FY= fat yield; PY= protein yield; LY= lactose yield; CSNY= casein yield; FP= fat percentage; PP= protein percentage; LP= lactose percentage; CSNP= casein percentage; CSNP:PP= ratio of casein to protein; CSNP:FP=ratio of casein to fat; CSNP:Calcium= ratio of casein to calcium content; SCS=somatic cell score; RCT= rennet coagulation time after rennet addition; K20= time to reach curd firmness of 20 mm after coagulation; A30= curd firmness at 30 min after rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time. Positive correlations are highlighted in green, while negative correlations are denoted in red. Strong correlations are depicted with intense colours, whereas weaker correlations are represented with faded shades.

Table 6.4 (continuation). Genetic correlations (below diagonal) and phenotypic correlations (above diagonal) with confidence interval (in brackets) between milk production, composition, and processability traits, for dairy sheep milked in mid and late lactation during the 2021-2022 season

Trait[1]	CSNP:PP	CSNP:FP	CSNP:Ca	Ca	Urea	SCS
MY	0.00	0.02	-0.10	0.01	0.14	-0.13
	(-0.13; 0.13)	(-0.09; 0.13)	(-0.22; 0.02)	(-0.11; 0.13)	(0.03; 0.25)	(-0.25; -0.01)
FY	-0.01	-0.34	-0.08	0.08	-0.06	-0.12
	(-0.13; 0.12)	(-0.43; -0.24)	(-0.20; 0.04)	(-0.06; 0.20)	(-0.18; 0.07)	(-0.22; 0.01)
PY	-0.02	0.08	-0.05	0.07	0.11	-0.13
	(-0.16; 0.10)	(-0.11; 0.25)	(-0.17; 0.07)	(-0.046; 0.20)	(-0.02; 0.23)	(-0.34; 0.12)
LY	0.01	0.03	-0.11	0.02	0.15	-0.16
	(-0.12; 0.14)	(-0.08; 0.14)	(-0.23; 0.01)	(-0.10; 0.13)	(0.03; 0.27)	(-0.27; -0.05)
CSNY	0.04	0.06	-0.06	0.11	0.12	-0.09
	(-0.09; 0.16)	(-0.05; 0.16)	(-0.19; 0.06)	(-0.01; 0.22)	(-0.01; 0.24)	(-0.22; 0.02)
FP	0.00	-0.72	0.00	0.19	-0.43	0.10
	(-0.12; 0.11)	(-0.77; -0.66)	(-0.11; 0.11)	(0.09; 0.30)	(-0.51; 0.34)	(0.00; 0.20)
PP	-0.07	0.16	0.28	0.29	-0.21	0.22
	(-0.19; 0.06)	(0.06; 0.27)	(0.15; 0.39)	(0.17; 0.41)	(-0.33; -0.09)	(0.11; 0.33)
LP	0.09	0.05	-0.22	0.05	0.17	-0.34
	(-0.04; 0.22)	(-0.07; 0.15)	(-0.34; -0.10)	(-0.07; 0.16)	(0.05; 0.29)	(-0.44; -0.24)
CSNP	0.20	0.18	0.25	0.36	-0.16	0.13
	(0.07; 0.32)	(0.07; 0.29)	(0.13; 0.37)	(0.25; 0.47)	(-0.28; -0.03)	(0.00; 0.26)
CSNP:PP		0.08	-0.01	0.17	0.13	-0.15
		(-0.03; 0.18)	(-0.12; 0.10)	(0.05; 0.28)	(0.02; 0.24)	(-0.26; -0.05)
CSNP:FP	0.00		0.10	0.04	0.30	0.05
	(-0.44; 0.42)		(-0.01; 0.20)	(-0.07; 0.14)	(0.20; 0.39)	(-0.06; 0.15)
CSNP:Ca	-0.12	0.22		-0.72	-0.11	0.13
	(-0.55; 0.43)	(-0.22; 0.58)		(-0.77; -0.65)	(-0.22; 0.00)	(0.02; 0.24)
Ca	0.20	0.03	-0.47		0.01	-0.05
	(-0.31; 0.60)	(-0.41; 0.47)	(-0.76; 0.09)		(-0.11; 0.13)	(-0.17; 0.06)

Table 6.4 (continuation). Genetic correlations (below diagonal) and phenotypic correlations (above diagonal) with confidence interval (in brackets) between milk production, composition, and processability traits, for dairy sheep milked in mid and late lactation during the 2021-2022 season.

Trait[1]	CSNP:PP	CSNP:FP	CSNP:Ca	Ca	Urea	SCS
Urea	-0.06	0.13	-0.20	-0.10		-0.04
	(-0.49; 0.41)	(-0.30; 0.57)	(-0.60; 0.28)	(-0.56; 0.41)		(-0.14; 0.08)
SCS	-0.22	0.18	0.06	0.04	0.04	
	(-0.63; 0.27)	(-0.29; 0.56)	(-0.42; 0.55)	(-0.46; 0.55)	(-0.42; 0.49)	
RCT	-0.28	0.21	0.32	-0.01	-0.15	0.30
	(-0.68; 0.25)	(-0.28; 0.63)	(-0.19; 0.70)	(-0.53; 0.51)	(-0.60; 0.36)	(-0.26; 0.73)
K20	-0.17	0.08	0.25	-0.23	0.00	0.28
	(-0.55; 0.36)	(-0.40; 0.47)	(-0.25; 0.65)	(-0.65; 0.33)	(-0.46; 0.43)	(-0.34; 0.64)
A30	0.18	-0.05	-0.16	0.17	0.04	-0.23
	(-0.39; 0.63)	(-0.49; 0.04)	(-0.59; 0.44)	(-0.40; 0.62)	(-0.43; 0.51)	(-0.67; 0.34)
ILCY	-0.04	-0.24	-0.08	0.40	-0.33	0.21
	(-0.49; 0.42)	(-0.65; 0.26)	(-0.53; 0.38)	(-0.08; 0.72)	(-0.72; 0.19)	(-0.34; 0.63)
HCT	-0.17	-0.01	0.03	-0.16	-0.12	-0.02
	(-0.60; 0.34)	(-0.50; 0.52)	(-0.49; 0.54)	(-0.60; 0.38)	(-0.60; 0.42)	(-0.00; -0.03)
pH	-0.08	0.13	-0.30	0.29	0.12	0.37
	(-0.50; 0.33)	(-0.34; 0.52)	(-0.63; 0.15)	(-0.19; 0.63)	(-0.32; 0.52)	(-0.13; 0.69)

[1] MY= milk yield; FY= fat yield; PY= protein yield; LY= lactose yield; CSNY= casein yield; FP= fat percentage; PP= protein percentage; LP= lactose percentage; CSNP= casein percentage; CSNP:PP= ratio of casein to protein; CSNP:FP=ratio of casein to fat; CSNP:Calcium= ratio of casein to calcium content; SCS=somatic cell score; RCT= rennet coagulation time after rennet addition; K20= time to reach curd firmness of 20 mm after coagulation; A30= curd firmness at 30 min after rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time. Positive correlations are highlighted in green, while negative correlations are denoted in red. Strong correlations are depicted with intense colours, whereas weaker correlations are represented with faded shades.

Table 6.4 (continuation). Phenotypic correlations with confidence interval (in brackets) between milk production, composition, and processability traits, for dairy sheep milked in mid and late lactation during the 2021-2022 season.

Trait[1]	RCT	K20	A30	ILCY	HCT	pH
MY	-0.21	-0.12	0.13	-0.12	0.12	-0.01
	(-0.48; 0.07)	(-0.24; 0.00)	(0.02; 0.25)	(-0.25; 0.02)	(-0.03; 0.25)	(-0.12; 0.12)
FY	-0.15	-0.13	0.14	0.18	0.07	-0.08
	(-0.28; -0.04)	(-0.25; -0.02)	(0.03; 0.26)	(0.05; 0.31)	(-0.08; 0.21)	(-0.20; 0.03)
PY	-0.11	-0.11	0.12	-0.02	0.13	0.00
	(-0.23; 0.01)	(-0.23; -0.01)	(0.00; 0.25)	(-0.17; 0.12)	(-0.01; 0.26)	(-0.12; 0.11)
LY	-0.19	-0.14	0.16	-0.13	0.11	0.00
	(-0.30; -0.07)	(-0.25; -0.02)	(0.04; 0.26)	(-0.26; -0.01)	(-0.03; 0.25)	(-0.12; 0.12)
CSNY	-0.11	-0.13	0.14	0.04	0.12	0.00
	(-0.24; 0.01)	(-0.24; -0.01)	(0.02; 0.26)	(-0.10; 0.17)	(-0.03; 0.26)	(-0.12; 0.12)
FP	0.01	-0.03	0.04	0.71	-0.06	-0.08
	(-0.09; 0.12)	(-0.14; 0.08)	(-0.06; 0.15)	(0.64; 0.76)	(-0.91; 0.08)	(-0.17; 0.04)
PP	0.28	0.08	-0.08	0.47	-0.01	0.08
	(0.16; 0.38)	(-0.03; 0.19)	(-0.20; 0.06)	(0.36; 0.57)	(-0.16; 0.15)	(-0.04; 0.20)
LP	-0.38	-0.27	0.29	-0.20	-0.10	-0.11
	(-0.48; -0.28)	(-0.37; -0.17)	(0.18; 0.40)	(-0.32; -0.06)	(-0.24; 0.05)	(-0.22; 0.00)
CSNP	0.21	0.02	-0.01	0.45	-0.03	0.07
	(0.08; 0.33)	(-0.10; 0.13)	(-0.14; 0.13)	(0.33; 0.56)	(-0.16; 0.12)	(-0.06; 0.18)
CSNP:PP	-0.22	-0.16	0.35	0.03	-0.08	0.03
	(-0.33; -0.09)	(-0.26; -0.04)	(0.25; 0.46)	(-0.12; -0.16)	(-0.20; 0.06)	(-0.09; 0.15)
CSNP:FP	0.13	0.08	-0.09	-0.46	0.08	0.14
	(0.01; 0.24)	(-0.02; 0.18)	(-0.20; 0.02)	(-0.57; -0.35)	(-0.06; 0.21)	(0.04; 0.25)
CSNP:Ca	0.33	0.26	-0.24	0.12	0.04	-0.09
	(0.22; 0.43)	(0.16; 0.36)	(-0.33; -0.12)	(-0.02; 0.24)	(-0.10; 0.17)	(-0.19; 0.03)
Ca	-0.16	-0.21	0.19	0.15	-0.06	0.08
	(-0.27; -0.05)	(-0.31; -0.10)	(0.08; 0.31)	(0.02; 0.28)	(-0.20; 0.08)	(-0.02; 0.19)

Table 6.4 (continuation). Genetic correlations (below diagonal) and phenotypic correlations (above diagonal) with confidence interval (in brackets) between milk production, composition, and processability traits, for dairy sheep milked in mid and late lactation during the 2021-2022 season.

Trait[1]	RCT	K20	A30	ILCY	HCT	pH
Urea	-0.13	-0.07	0.10	-0.47	-0.02	0.15
	(-0.24; -0.02)	(-0.17; 0.03)	(-0.02; 0.20)	(-0.57; -0.36)	(-0.17; 0.11)	(0.04; 0.26)
SCS	0.28	0.20	-0.23	0.32	-0.02	0.16
	(0.17; 0.39)	(0.10; 0.30)	(-0.33; -0.12)	(0.19; 0.44)	(-0.003; -0.03)	(0.05; 0.27)
RCT		0.85	-0.86	0.04	0.20	0.33
		(0.81; 0.88)	(-0.88; -0.82)	(-010; 0.16)	(0.07; 0.34)	(0.21; 0.43)
K20	0.74		-0.89	-0.05	0.11	0.20
	(0.41; 0.90)		(-0.91; -0.86)	(-0.20; 0.08)	(-0.02; 0.25)	(0.07; 0.30)
A30	-0.72	-0.79		0.05	-0.17	-0.30
	(-0.90; -0.36)	(-0.92; -0.43)		(-0.08; 0.18)	(-0.31; -0.04)	(-0.40; -0.18)
ILCY	-0.01	-0.16	0.13		0.04	0.19
	(-0.49; 0.46)	(-0.62; 0.37)	(-0.38; 0.59)		(-0.10; 0.18)	(0.06; 0.34)
HCT	0.40	0.28	-0.40	-0.14		0.26
	(-0.10; 0.76)	(-0.26; 0.67)	(-0.74; 0.18)	(-0.60; 0.38)		(0.11; 0.40)
pH	0.57	0.40	-0.50	0.26	0.50	
	(0.18; 0.80)	(-0.10; 0.70)	(-0.77; -0.01)	(-0.20; 0.64)	(0.07; 0.77)	

[1] MY= milk yield; FY= fat yield; PY= protein yield; LY= lactose yield; CSNY= casein yield; FP= fat percentage; PP= protein percentage; LP= lactose percentage; CSNP= casein percentage; CSNP:PP= ratio of casein to protein; CSNP:FP=ratio of casein to fat; CSNP:Calcium= ratio of casein to calcium content; SCS=somatic cell score; RCT= rennet coagulation time after rennet addition; K20= time to reach curd firmness of 20 mm after coagulation; A30= curd firmness at 30 min after rennet addition; ILCY= individual laboratory cheese yield; HCT= heat coagulation time. Positive correlations are highlighted in green, while negative correlations are denoted in red. Strong correlations are depicted with intense colours, whereas weaker correlations are represented with faded shades.

6.4 Discussion

The level of milk production for this population of dairy sheep in the studied season has been previously discussed, with lactation curves modelled and total produced yields predicted (Marshall et al. 2023). The relatively low milk production was suggested to be mainly a consequence of low pasture availability. The average processability characteristics of milk from the individual ewes during the once-a-day milking period have also been discussed in another study, which evaluated the effect of animal factors on processability traits (Marshall et al. 2024). Overall, the milk coagulation properties of this flock were within the range reported for dairy sheep, and individual cheese yield was high. However, milk heat stability of individual ewes could not be compared with other flocks due to a lack of studies.

6.4.1 Variances, heritability, and repeatability

A wide range of heritability values for yield traits has been reported for other populations of dairy sheep across the globe (0.06-0.54) (Mavrogenis and Papachristoforou 2000; Barillet 2007; Puledda et al. 2017). Differences in estimates of heritability and repeatability between studies are mainly due to different breeds and population structures but can also be a consequence of sample size. The overall low repeatability values reported here is due to the lower combined additive genetic and permanent environmental sources of variation in proportion to other temporary environmental influences (Dohm et al. 2002).

The moderate estimates of heritability for MY, FY, PY, and LY reported here suggest that there could be a significant response through selective breeding on these traits (Lopez-Villalobos 2012). The heritability estimates for MY, FY, PY, and LY were higher than the range reported for other populations of East-Friesian sheep (0.09-0.25) (Horstick et al. 2002; Hamann et al. 2004; De Vries et al. 2005).

The heritability values for FP, PP, LP are usually higher and can be twice the value of the heritability values for FY, PY, LY in dairy cows (Lopez-Villalobos 2012; Lembeye et al. 2016). However, few studies have reported heritability estimates for both yield and milk composition traits in dairy sheep, and the pattern is not consistent (Barillet 2007; Sánchez-Mayor et al. 2019; Mucha et al. 2022). In the present study, the heritability values for PP and LP were higher than but not twice the heritability values for PY and LY. Whereas the

heritability for FP (0.19) was lower than the heritability of FY (0.33). This apparent contradiction could be due to FP being highly affected by the variation of feed (Morand-Fehr et al. 2007), and, in this study, the nutritional value of the pasture varied significantly throughout the season.

Overall, heritability estimates for processability traits were lower than heritability values for milk yield and composition traits. Low to moderate heritability estimates for MCP have also been previously reported (Sánchez-Mayor et al. 2019). Amongst the MCP traits, RCT had the highest heritability (0.27), and K20 had the lowest (0.23), agreeing with the pattern reported by Bittante et al. (2017). The heritability for RCT estimated here also goes in agreement with the findings gathered from earlier studies on dairy cows (Bittante et al. 2012)

Lower heritability estimates for milk pH (0.15- 0.20) have been reported by Bittante et al. (2017) and Puledda et al. (2017) for Sarda sheep in Italy, but these studies also reported low heritability for milk composition traits. Higher heritability values of 0.34 and 0.46 for milk pH have been reported for Spanish Churra (Pelayo et al. 2021) and Assaf sheep (Sánchez-Mayor et al. 2019), respectively.

6.4.3. Genetic and phenotypic correlations

Genetic and phenotypic correlations, in general, followed a similar trend. Differences between phenotypic and genetic correlations could be explained by the relationship between genetic and environmental effects, and certain traits have environmental effects that act in the opposite direction to the genetic effects (Sodini et al. 2018).

The high correlation values among milk yield traits were expected (Lopez-Villalobos et al. 2012; Scholtens et al. 2018). In addition, the high correlations among MCP found in the present study agree with findings for dairy cows and ewes (Bittante et al. 2012; Pelayo et al. 2021).

The weak correlation between MY and FP ($r_G = -0.07$) and PP ($r_G = -0.11$) reported here could be due to the overall low milk production of the flock in the season, a consequence of the low pasture availability. Stronger negative correlations between milk yield and composition traits have been widely reported for dairy sheep (Othmane et al 2002, Barillet et al. 2007; Sánchez-Mayor et al. 2019).

The yield traits MY, FY, PY, and LY were negatively correlated with RCT and K20 ($-0.24 \leq r_G \leq -0.11$), and positively correlated with A30 ($0.10 \leq r_G \leq 0.15$). Although the estimated correlations between milk yield traits and MCP were weak to moderate, and the confidence intervals were wide, the trends suggest that the improvement of these dairy sheep on the traditional milk production traits is likely to result in an indirect improvement of the quality of milk for processing into cheese.

It is also worth noting that the negative correlation observed between yield traits and SCS ($-0.28 \leq r_G \leq -0.20$) and pH ($-0.22 \leq r_G \leq -0.18$) could imply that worse milk quality was associated with lower producing animals, which consequently worsened milk rennet coagulation properties. However, the negative correlation between MY and ILCY ($r_G = -0.23$) indicates that improving milk yield could entail a penalty in cheese quantity, which has also been previously noted (Othmane et al. 2002). This is further supported by the strong-moderate positive correlations between ILCY and composition traits FP ($r_G = 0.58$) and PP ($r_G = 0.43$).

Although milk yield traits had contrasting correlations with quality (MCP) and quantity (ILCY) traits relevant to cheese production, the lack of correlation between ILCY and MCP makes it challenging to draw conclusions regarding the relationship between cheese quality and quantity. It is possible that the methodology used for estimating ILCY hindered the assessment of true correlations with MCP. In agreement, others have also estimated poor correlations between ILCY and MCP (Sánchez-Mayor et al. 2019; Pelayo et al. 2021). Therefore, it is recommended that future research use more sophisticated methods for estimating cheese yield (Cipolat-Gotet et al. 2016).

The positive moderate genetic correlations between HCT and RCT ($r_G = 0.40$), as well as K20 ($r_G = 0.28$), alongside the negative moderate genetic correlation between HCT and A30 ($r_G = -0.40$), suggests that dairy sheep flocks could be specifically oriented towards either milk production for cheese manufacture or to better milk heat stability for processing into milk powder, infant formula, or UHT beverages. However, there needs to be a way for farmers to capture the added value on milk processability (Cole et al. 2023).

There were no genetic correlations between FP and MCP. Whereas the positive moderate correlations found between RCT and PP ($r_G = 0.34$) and CSNP ($r_G = 0.23$) indicates that a high content of protein and casein were associated with longer time to reach coagulation after rennet addition. In agreement, Ikonen et al. (2004) and Vacca et al. (2019) also

reported lower protein content to be correlated to short RCT in dairy cows and in dairy sheep. The higher rennet stability of high-protein milks may be due to partitioning of more calcium (and other divalent ions) within micelles rather than in the serum (Grimley et al. 2009). Lower ionic calcium in the serum would decrease salt-bridging effects and thereby slow coagulation. Indeed, the ratio of casein to calcium (CSNP:Calcium), which is opposite to soluble calcium availability, had a positive correlation with RCT ($r_G = 0.32$) and K20 ($r_G = 0.25$).

Lactose percentage had strong moderate negative genetic correlations with RCT ($r_G = -0.45$) and K20 ($r_G = -0.33$), and positive correlation with A30 ($r_G = 0.34$), agreeing with others (Vacca et al. 2019). Lactose percentage might be indirectly associated with MCP because lactose is an osmotic regulator in the mammary gland (Fox et al. 2015, Vacca et al. 2019). In case of infections in the mammary gland, LP and the content of milk salts in the soluble phase can be affected (Costa et al. 2019; Antanaitis et al. 2021). The strong moderate negative correlation between LP and SCS ($r_G = -0.47$) has also been widely reported (Shuster et al. 1991; Albenzio et al. 2004; Costa et al. 2019), supporting the relationship between LP and mammary gland health. In addition, it has also been proposed that lactose can have a direct effect on casein rennet-induced gelation by strengthening the hydrophobic interactions between the casein micelles (Niki and Motoshima 2006).

Furthermore, the moderate positive genetic correlation between SCS and RCT ($r_G = 0.30$) and K20 ($r_G = 0.28$), and the negative correlation with A30 ($r_G = -0.23$), indicate that elevated SCS levels were linked to prolonged rennet coagulation times and softer curd. Moreover, higher SCS levels correlated with increased milk pH ($r_G = 0.37$), agreeing with other reports (Ikonen et al. 2004; Pazzola et al. 2018). Milk of high SCS has been reported to have high plasmin level, which leads to degradation of casein, impairing coagulation (Albenzio et al. 2004). Consistent with this, SCS had a negative correlation with the casein to protein ratio ($r_G = -0.22$).

Higher milk pH was associated with longer rennet coagulation times and softer curd, seen by the strong moderate positive genetic correlations with RCT ($r_G = 0.57$) and K20 ($r_G = 0.40$), and a negative correlation with A30 ($r_G = -0.50$), agreeing with others (Battacone et al. 2005; Puledda et al. 2017; Pazzola et al. 2018). The same trend has been reported for dairy cows (Ikonen et al. 2004; Bonfatti et al. 2014). Higher milk pH is known to impair

the action of the enzyme chymosin and increase the stability of casein micelles (Bencini 2002; Pirisi et al. 2007).

Despite having obtained some large confidence intervals due to small sample size, the trends identified in the present study suggest that poorer MCP were associated with lower mammary health indicated by higher SCS, higher pH, and lower LP, which is consistent with previous research (Battacone et al. 2005; Pazzola et al. 2018; Correddu et al. 2022). Additionally, a higher ratio of casein to calcium i.e. lower availability of soluble calcium was associated with poorer MCP. Higher yields of milk, fat, protein, and lactose were associated, but to a smaller degree, with better MCP.

Considering the impracticality of measuring processability traits within recording schemes, the lower heritability values, and that the usual payment systems in dairy industries reward producers for high yields of fat and protein, exemplified by the breeding objective of New Zealand dairy cattle that focuses on maximising the efficient conversion of feed into milk solids (Lopez-Villalobos 2012), improving dairy sheep flocks, like the one studied, for higher fat and protein yields is proposed, which could potentially lead to an indirect improvement in processability for cheesemaking. Additionally, a penalty on high somatic cell score is recommended to improve sheep milk quality for processing.

However, it is unheard that manufacturers of sheep milk cheeses in New Zealand encounter issues with non-coagulating milk. Conversely, manufacturers of sheep milk powder may be facing challenges with the heat stability of sheep milk (Pan 2023), highlighting a need for deeper investigation into dairy sheep genetics for milk heat stability.

From a technological perspective, exploring genetic correlations between milk production traits and additional traits such as the profile of protein fractions, fatty acids, and soluble minerals could deepen the understanding of the mechanism behind the milk coagulation process (Duchemin et al. 2020), and further validate findings from experimental studies. However, the selection of traits for inclusion in a selection index should consider the feasibility of measurement, economic value, and heritability (Shook 1989). Alternatively, processability traits can be measured in a training population, enabling genomic predictions that indicate milk processing quality (Jones and Wilson 2022).

Ultimately, establishing selection indexes for dairy sheep is important as it optimizes milk production from fewer ewes, improving farmer profitability, whilst significantly reducing

the use of natural resources, indirectly contributing to farm sustainability. Research is also needed on the genetic correlations between milk production and sustainability and resilience traits in dairy sheep in New Zealand (Barillet 2007; Mucha et al. 2022).

It is crucial to preserve the knowledge gained from small farms like the one in this study, as pedigree information for dairy sheep is often difficult to maintain or access. Small farms also contribute to overall genetic diversity and resilience within the dairy sheep farming community in New Zealand and should not be overlooked. Further genome-wide association studies are underway and will allow further understanding of the genetic architecture of milk processability traits. Additionally, genomic selection could increase accuracy and facilitate dairy sheep improvement on traits that are not easily measured.

6.5 Conclusion

The results of the present study suggest that genetic improvement of this flock of dairy sheep on the traditional traits of fat yield, protein yield, and lower somatic cell count could also lead to an indirect improvement of milk quality for cheese-making. However, selecting for improved cheese-making aptitude might undesirably worsen milk heat stability, potentially evidencing the need for a separate selection index for dairy sheep flocks destined to milk powder production, which is important for export revenue. It is recommended that larger studies involving more dairy sheep flocks are conducted to validate the present results before developing a selection index for the dairy sheep industry.

References

Albenzio M, Caroprese M, Santillo A, Marino R, Taibi L, Sevi A. 2004. Effects of somatic cell count and stage of lactation on the plasmin activity and cheese-making properties of ewe milk. Journal of Dairy Science. 87: 533−542.

Antanaitis, R., Juozaitienė, V., Jonike, V., Baumgartner, W., Paulauskas, A. (2021). Milk lactose as a biomarker of subclinical mastitis in dairy cows. Animals , 11:(6), 1736.

Barillet F. 2007. Genetic improvement for dairy production in sheep and goats. Small Ruminant Research. 70: 60−75.

Bencini R and Pulina G. 1997. The quality of sheep milk: A review. Australian Journal of Experimental Agriculture. 37: 485−504.

Bencini R. 2002. Factors affecting the clotting properties of sheep milk. Journal of the Science of Food and Agriculture. 82: 705−719.

Bittante G, Cipolat-Gotet C, Pazzola M, Dettori M, Vacca G, Cecchinato A. 2017. Genetic analysis of coagulation properties, curd firming modeling, milk yield, composition, and acidity in Sarda dairy sheep. Journal of Dairy Science. 100: 385−394.

Bittante, G., Penasa, M., Cecchinato, A. (2012). Invited review: Genetics and modeling of milk coagulation properties. Journal of Dairy Science. 95: 6843−6870.

Cheng H, Fernando RL, and Garrick DJ. 2018 JWAS: Julia implementation of whole-genome analysis software. Proceedings of the World Congress on Genetics Applied to Livestock Production. 11:859. Auckland, New Zealand.

Correddu, F., Gaspa, G., Cesarani, A., Macciotta, N. P. P. (2022). Phenotypic and genetic characterization of the occurrence of noncoagulating milk in dairy sheep. Journal of Dairy Science. 105: 6773−6782.

Costa A, Lopez-Villalobos N, Visentin G, De Marchi M, Cassandro M, Penasa M. 2019. Heritability and repeatability of milk lactose and its relationships with traditional milk traits, somatic cell score and freezing point in Holstein cows. Animal. 13: 909−916.

De Vries F, Hamann H, Drögemüller C, Ganter M, Distl O. 2005. Analysis of associations between the prion protein genotypes and production traits in East Friesian milk sheep. Journal of Dairy Science. 88: 392−398.

Dohm MR. 2002. Repeatability estimates do not always set an upper limit to heritability. Functional Ecology. 16: 273−280.

Downie-Melrose, K. 2014. A Bio-economic feasibility study of sheep dairy systems in Canterbury. Lincoln University, Lincoln, New Zealand.

Flint APF, and Woolliams JA. 2008. Precision animal breeding. Philosophical Transactions of the Royal Society B: Biological Sciences. 363: 573−590.

Fox PF, Uniacke-Lowe T, McSweeney PLH and O'Mahoni JA. 2015. Dairy chemistry and biochemistry. Springer International Publishing, Cham, Switzerland.

Grimley, H., Grandison, A., Lewis, M. 2009. Changes in milk composition and processing properties during the spring flush period. Dairy Science and Technology. 89: 405−416.

Gullery L. 2021. Dairy`s other export not sheepish about growth. https://www.stuff.co.nz/business/farming/dairy/126515904/dairys-other-export-not-sheepish-about-growth. (accessed on 23 May 2024).

Hamann H, Horstick A, Wessels A, Distl O. 2004. Estimation of genetic parameters for test day milk production, somatic cell score and litter size at birth in East Friesian ewes. Livestock Production Science. 87: 153−160.

Horstick A, Hamann H, Distl O. 2002. Estimation of genetic parameters for daily milk performance of East Friesian milk sheep by random regression models. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France, August, 2002. Session 1. Pp. 1−4.

Ikonen T, Morri S, Tyrisevä A-M, Ruottinen O, Ojala M. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. Journal of Dairy Science. 87: 458−467.

Jones HE, and Wilson PB. 2022. Progress and opportunities through use of genomics in animal production. Trends in Genetics. 38: 1228−1252.

Lees N, and Lees I. 2018. Competitive advantage through responsible innovation in the New Zealand sheep dairy industry. International food and agribusiness management review 21: 505−524.

Lembeye F, López-Villalobos N, Burke J, Davis S. 2016. Estimation of genetic parameters for milk traits in cows milked once-or twice-daily in New Zealand. Livestock Science. 185: 142−147.

Lopez-Villalobos N. 2012. Analysing the genetic basis of milk production traits. Animal Science Reviews: 65−78.

Marshall AC, Lopez-Villalobos N, Loveday SM, Ellis A, McNabb W. 2023. Modelling lactation curves for dairy sheep in a New Zealand flock. Animals. 13: 349.

Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024. Animal factors affecting the cheese-making properties and the heat coagulation time of milk from dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. Published online: 27 Mar 2024: 1−18.

Mavrogenis A, Papachristoforou C. 2000. Genetic and phenotypic relationships between milk production and body weight in Chios sheep and Damascus goats. Livestock Production Science. 67: 81−87.

McMahon DJ, Brown RJ. 1982. Evaluation of Formagraph for comparing rennet solutions. Journal of Dairy Science. 65: 1639−1642.

Meteorological Service of New Zealand Ltd. 2022. Available online: https://www.metservice.com/towns-cities/locations/masterton/past-weather (accessed on 5 September 2022).

MPI-Ministry of Primary Industries, and Massey University. 2020. New Zealand Sheep Dairy Survey. https://www.mpi.govt.nz/dmsdocument/43183-Sheep-Dairy-Survey-Report-July-2020 (accessed on 04 May 2024).

Morand-Fehr P, Fedele V, Decandia M, Le Frileux Y. 2007. Influence of farming and feeding systems on composition and quality of goat and sheep milk. Small Ruminant Research. 68: 20−34.

Mucha S, Tortereau F, Doeschl-Wilson A, Rupp R, Conington J. 2022. Animal Board Invited Review: Meta-analysis of genetic parameters for resilience and efficiency traits in goats and sheep. Animal. 16: 100456.

Niki R, and Motoshima H. 2006. Influence of lactose on viscoelastic properties of acid-induced casein micelle gels: the secondary roll of lactose in yoghurt production. Milchwissenschaft 61: 411−415.

New Zealand Sheepbreeders Association. 2024 a. Dairymeade breed information. https://nzsheep.co.nz/dairymeade-nz/ (accessed on 23 May 2024).

New Zealand Sheepbreeders Association. 2024 b. Southern Cross breed information. https://nzsheep.co.nz/southern-cross/ (accessed on 23 May 2024).

New Zealand Sheepbreeders Association. 2024 c. Zealandia breed information. https://nzsheep.co.nz/zealandia/ (accessed on 23 May 2024).

Pazzola M, Cipolat-Gotet C, Bittante G, Cecchinato A, Dettori ML, Vacca GM. 2018. Phenotypic and genetic relationships between indicators of the mammary gland health status and milk composition, coagulation, and curd firming in dairy sheep. Journal of Dairy Science. 101: 3164−3175.

Pelayo R, Gutiérrez-Gil B, Garzón A, Esteban-Blanco C, Marina H, Arranz J. 2021. Estimation of genetic parameters for cheese-making traits in Spanish Churra sheep. Journal of Dairy Science. 104: 3250−3260.

Peterson S, Prichard C 2015. The sheep dairy industry in New Zealand: A review. New Zealand Society of Animal Production. Pp. 119−126.

Pirisi A, Murgia A, Scintu M. 1994. Estimate of Pecorino romano and Pecorino sardo cheese yield from the protein and fat contents in sheep milk [Latium, Sardinia]. Scienza e Tecnica Lattiero-Casearia (Italy).

Puledda A, Gaspa G, Manca MG, Serdino J, Urgeghe PP, Dimauro C, Negrini R, Macciotta NPP. 2017. Estimates of heritability and genetic correlations for milk coagulation properties and individual laboratory cheese yield in Sarda ewes. Animal. 11: 920−928.

Sánchez-Mayor M, Pong-Wong R, Gutiérrez-Gil B, Garzón A, De la Fuente L, Arranz J. 2019. Phenotypic and genetic parameter estimates of cheese-making traits and their relationships with milk production, composition and functional traits in Spanish Assaf sheep. Livestock Science. 228: 76−83.

SAS. Statistical Analysis System, Version 9.4; SAS Institute Inc.: Cary, NC, USA, 2004.

Scale up. 2022. Government supports innovative dairy sheep sector to scale up. https://www.beehive.govt.nz/release/government-supports-innovative-dairy-sheep-sector-scale (accessed on 23 May 2024).

Scholtens MR, Peterson S, Gunson K, Gunson A, Lopez-Villalobos N. 2018. Genetic evaluation of dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. 61: 81−91.

Shook GE. 1989. Selection for disease resistance. Journal of Dairy Science 72: 1349−62.

Shuster D, Harmon R, Jackson J, Hemken R. 1991. Suppression of milk production during endotoxin-induced mastitis. Journal of Dairy Science. 74: 3763−3774.

Sodini SM, Kemper KE, Wray NR, Trzaskowski M. 2018. Comparison of genotypic and phenotypic correlations: Cheverud's conjecture in humans. Genetics. 209: 941−948.

Vacca G, Cipolat-Gotet C, Paschino P, Casu S, Usai M, Bittante G, Pazzola M. 2019. Variation of milk technological properties in sheep milk: Relationships among composition, coagulation and cheese-making traits. International Dairy Journal. 97: 5−14.

Chapter 7

Genome-wide association study for milk production and composition, milk coagulation properties, and milk protein profile in dairy sheep from a New Zealand flock

This chapter has been submitted for publication elsewhere and is being reviewed:

Marshall AC, Vigolo V, De Marchi M, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2025. Genome-wide association study for milk production and composition, milk coagulation properties, and milk protein profile in dairy sheep from a New Zealand flock. Animal Biotechnology.

Abstract

The objective of this study was to perform genome-wide association analysis for milk production and composition, milk coagulation properties (MCP), and milk protein profile in dairy sheep from a New Zealand flock. After quality control, 45,801 single nucleotide polymorphisms (SNPs) were included in the analysis, 147 ewes, and 470 individual records. A total of 87 SNPs and 55 candidate genes were found across Ovis aries autosomes (OAR) 2, 3, 6, 16, 18, 20, 25, and 26. Of particular importance, were the candidate genes PDZRN4 and CNTN1 for milk yield and α-lactalbumin, TBK1 for casein and α-lactalbumin, SNTG2 for calcium, CTSL for ratio of casein to protein, DCK for milk yield and α_{s2}-casein, ADAMTS20 and LRRK2 for α-lactalbumin, BMP2K for α_{s1}- and α_{s2}-caseins, and EMB for milk pH. No SNPs were found in casein genes, LALBA or PAEP. Only one SNP was significant for MCP, and overall, the genetic architecture of MCP was similar to that of ratio of casein to calcium, pH, lactose, and the ratio of casein to protein. Further studies with larger flocks and with genomic imputation are required to validate the findings of this study before incorporating markers or genes into breeding programs.

Keywords

Sheep milk; Dairy sheep; Milk production, Milk composition; Protein profile; Genome-wide association.

7.1 Introduction

The New Zealand dairy sheep industry is still in its early stages and necessitates improved animal genetics for dairy traits (Lees and Lees 2018), but also that are fit for the New Zealand climate and farming systems, which are mainly pasture-based. Genomic information could play a crucial role in accelerating genetic gains and improving the accuracy of breeding value estimations. Furthermore, identifying specific genetic markers that significantly impact dairy traits would provide an additional tool for selection (Johnsson 2023). For instance, a few single marker tests are already commercially available and used in New Zealand meat sheep selection for increased fecundity, disease resistance, and meat production such as meat yield and yellow fat (McEwan 2009).

Genome-wide association studies (GWAS) are becoming increasingly popular in animal production to provide insights into the genetic architecture of important traits for

production, sustainability, or animal resilience. In animal breeding, GWAS help identify markers like single nucleotide polymorphisms (SNPs) associated with quantitative traits, which are controlled by multiple genes located in quantitative trait loci (QTLs), making them polygenic. Some GWAS have been performed for dairy sheep across the globe on milk production and composition (Garcia-Gamez et al. 2012; Moioli et al. 2015; Sutera et al. 2019; Li et al. 2020; Mi et al. 2021), and somatic cell score (Sutera et al. 2021).

The findings of GWAS vary across studies in dairy sheep, indicating that knowledge in this field is still building. This variation is influenced by the different genetic architectures of the various breeds and populations studied. However, discrepancies within breed are also reported. For protein content in the milk of Churra sheep, a region on *Ovis aries* autosome (OAR) 6 near the casein cluster was suggested to influence protein percentage (Diez-Tascon et al. 2001), while others have pointed to OAR3 as being more significant (Gutiérrez-Gil et al. 2009; García-Gámez et al. 2012). In a non-selected dairy sheep breed (Altamurana), OAR3 was also the most relevant for protein percentage (Moioli et al. 2015). In contrast, OAR1 and OAR7 were important for protein percentage in Valle del Belice sheep (Sutera et al. 2019).

In New Zealand, a recent study found significant SNP associations with milk yield in dairy sheep (Costilla et al. 2023), in addition to studies on dairy goats (Scholtens et al. 2020) and dairy cows (Ariyarathne et al. 2021; Jayawardana et al. 2023). Additionally, to date, GWAS for sheep milk processability traits have been performed mainly in Mediterranean countries (Gaspa et al. 2016; Marina et al. 2021; Gaspa et al. 2022b; Gaspa et al. 2022a). Other genomic studies have targeted the casein genes (Noce et al. 2016) or single genes such as PRLR, GHR, and GHRHR (Dettori et al. 2020; Pazzola et al. 2021) with the use of PCR. No GWAS have yet been performed on New Zealand dairy sheep for milk coagulation properties (MCP), which provide information on the quality of milk for processing into cheese (McMahon and Brown 1982; Pazzola 2019).

Furthermore, the protein profile of milk (proportions of α_s-, α_{s2}-, β-, and κ-caseins, and α-lactalbumin and β-lactoglobulin in total protein), can influence the technological (Wedholm et al. 2006) and functional properties of milk (Mohapatra et al. 2019). While GWAS on protein profile have been performed on dairy cows (Dadousis et al. 2017b; Zhou et al. 2019), studies are lacking in dairy sheep. It is well known that the genes CSN1S1, CSN2, CSN1S2, and CSN3 encode for the four casein fractions and contribute

to their high protein polymorphism. The corresponding genes for whey proteins are LALBA and PAEP, which encode for α-lactalbumin and β-lactoglobulin, respectively (Caroli et al. 2009; Selvaggi et al. 2014). Genetic variation in these genes leads to different levels of protein expression, and consequently different physico-chemical properties of milk (Amalfitano et al. 2022). However, genetic variations in the milk protein profile not only are a result of these protein-encoding genes, but also influenced by the broader polygenic background of the animal (Bobe et al. 1999). For example, the molecular mechanism and factors controlling casein phosphorylation are still largely unknown (Fang et al. 2019).

Considering these, this study aimed to perform genome-wide analysis to identify associated SNPs and candidate genes potentially affecting milk yield and composition, milk coagulation properties, and the content of protein fractions in dairy sheep milk, and thus provide a better understanding of the genetic architecture controlling these traits.

7.2 Materials and methods

7.2.1 Animals and milk samples

This study was conducted at a commercial dairy sheep farm in Masterton, Wairarapa, New Zealand. Ethics approval was obtained for this study (Massey University Animal Ethics Committee-Protocol 21/45). The breed's development started in 1996, initially using Coopworth and Border Leicester dams from New Zealand and semen from European East Friesian sires. The resulting progenies were backcrossed with East Friesian sires. The farm has been using the self-replacement of ewes and rams over the past 12 years. In this dairy sheep breed, two colour variants are visually distinguished, white or black sheep. The small flock of around 170 milking ewes is traditionally managed on a pasture-based system with limited supplementation, with selection based mainly on temperament, dairy conformation and prolificacy. The age structure of the flock was 15% first-, 28% second-, 23% third- and 34% ≥fourth-parity ewes. Black ewes constituted 33% of the flock, while the remaining 67% were white. The median lambing date of the flock was the 20th of August 2021. Machine milking for cheese production started after the lamb suckling period, which lasted 57 days on average. A total of 470 test-day records from once-a-day milking were gathered from 01 November 2021 until 31 January 2022, from 147 ewes (50 to 182 days in milk), and this dataset was used for GWAS. A minimum of 2 test-day

records were obtained from each ewe. Information on pedigree, lambing date, litter size, and age of animals were supplied to the study. The average inbreeding coefficient, obtained from SNP & Variation Suite (SVS 8.8) software,of the whole flock was 4%. The average inbreeding coefficient of the group of animals with some level of inbreeding was 9%.

7.2.2 Milk yield and composition

Milk yield from individual ewes on a test-day was recorded from the total volume in the individual test buckets, and individual milk samples for compositional and processability analyses were taken. The milk samples were immediately refrigerated after collection and sodium azide (to final concentration of 0.025% w/w) was added for better preservation. All subsequent analyses were performed within three days following the sample collection.

Milk samples were analysed by Milk Test NZ Ltd (Hamilton, NZ) using a Combi FOSS instrument (Foss Analytics, Hilleroed, Denmark). The composition analysis included percentages of fat, protein, casein, lactose, and SCC (cells/mL) converted into somatic cell scores (SCS) using a Log_2 transformation. The analyses for percentage of casein and urea (mg/100mL) were performed using a Fourier-transform Infrared (FTIR) milk-analyser MilkoScan FT6000 (Foss Analytics) calibrated for sheep milk samples. The ratio of casein to protein (CSN:PP) is the casein content divided by the protein content. Total calcium content (mg/100 mL) was analysed by a contract laboratory (Massey University Nutrition Lab) using the Arsenazo III method (Randox reagent kit Ca8309) and the RX Daytona Plus clinical analyser. The ratio of casein to calcium (CSN:Ca) was calculated as casein (%) divided by calcium content (mg/100 mL), multiplied by 100.

7.2.3 Milk coagulation properties

The traditional milk coagulation properties of individual fresh milk samples were measured using a Formagraph instrument (Foss Analytics). The traits included rennet coagulation time (RCT), time to reach curd firmness of 20 mm after start of coagulation (K20), and curd firmness at 30 min after rennet addition (A30) (McMahon and Brown 1982). The preparation of samples was described in (Marshall et al. 2024c, Chapter 4).

7.2.4 Protein profile by RP-HPLC

Protein profile analyses (contents of κ-CN, α_{s1}-CN, α_{s2}-CN, β-CN, α-LA, and β-LG in total protein) were obtained by reverse-phase high performance liquid chromatography (RP-HPLC). The sample preparation method and instrument set up specifications were performed as per method of (Bobe et al. 1998) and (Bonfatti et al. 2008), respectively. The HPLC setup comprised an Agilent 1260 Infinity II LC system (Agilent Technologies) equipped with a quaternary pump (Agilent 1260 Infinity II, G7111B) and a diode array detector (Agilent 1260 Infinity II, G7115A) (Vigolo et al. 2022). The results were expressed as area (mAU) underneath the peaks of κ-CN, α_{s1}-CN, α_{s2}-CN, β-CN, α-LA, and β-LG, which represents the quantity of each protein fraction. A strong linear relationship ($r^2 > 0.90$) was observed between protein concentration in milk (g/L) and combined peak areas of protein fractions (total area). The percentage of each protein in total protein was calculated as the area of the respective fraction divided by the total area.

7.2.5 Genotyping and quality control

A total of 323 ewes, which included 169 ewes and 154 ewe lambs, and 6 rams had ear tissue samples collected for DNA extraction and scanning using an iScan® at the Equine Parentage Testing Lab (Massey University, Palmerston North). Genotype information was obtained using the OvineSNP50 Beadchip array (Illumina, San Diego, CA) with a medium-density SNP panel (50k SNPs). A total of 64,734 SNPs was obtained for quality control using the SNP & Variation Suite (SVS 8.8) software. In the filtering process, genomic records were removed for 23 animals with a call rate < 95% across all the SNPs, from which 4 animals were ewes that had phenotype records. Also, SNPs with > 5% missing genotypes across all individuals (call rate < 95%), and that had a significant deviation from the Hardy-Weinberg equilibrium threshold of $p < 10^{-6}$ or that had minor allele frequency < 1% were also removed. After these quality control edits, a total of 306 animals with SNPs (including 147 ewes with phenotypes), and 45,801 SNPs remained for association analyses.

Coat colour was identified as the first principal component (PC1) that explained >50% variation in the analysis and included as a fixed effect in the GWAS model described further ahead, the scatter plot is provided in Appendix 3 Figure 6.

7.2.6 Statistical analyses

Descriptive statistics for milk production and composition, milk coagulation properties, and contents of κ-CN, α_{s1}-CN, α_{s2}-CN, β-CN, α-LA, and β-LG (in total protein) were obtained using the MEANS procedure of SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

7.2.7 Genome-wide association analyses

The phenotypic traits were pre-corrected for fixed effects using the single-trait animal repeatability model in the ASReml 4.1 software package (Gilmour et al. 2015). Detailed number of animals in the pedigree file for ASReml is included in Appendix 3 Table 4. The model included the fixed effects of ewe coat colour (black or white), litter size (1 or ≥ 2 lambs), parity number (1st, 2nd, 3rd or ≥ 4th parity). Covariates included in the model were the effect of time (or days in milk), quadratic effect of time, and deviation from the median lambing date of the flock. The random effect of ewe permanent environment effect, and the random residual were included.

Principal component analyses for population stratification correction were performed in SVS software (SVS 8.8). PC1 was highly correlated (r = 0.95) with the coat colour effect and therefore PCs were not included, instead, the genomic relationship matrix was used for adjustment of population structure.

The genome-wide association study (GWAS) was performed using a mixed linear model in the GCTA software package (Yang et al. 2011). The following model was fitted for each trait:

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{X}\boldsymbol{\beta} + \mathbf{g} + \mathbf{e}$$

where $\mathbf{y}$ was the vector of pre-corrected phenotypes for each ewe, $\boldsymbol{\mu}$ was the vector of overall mean, $\boldsymbol{\beta}$ was the vector of each SNP fixed effect (additive SNP effect), and $\mathbf{X}$ was the incidence matrix of $\boldsymbol{\beta}$ to $\mathbf{y}$ with the SNPs genotypes of BB, AB or AA, respectively, $\mathbf{g}$

was the random additive polygenic effect (the accumulated effect of all SNPs) and **e** was the random residual error. The assumptions for the model were: $\mathbf{g} \sim N(0, \mathbf{G}\sigma_g^2)$, where **G** was the genomic relationship matrix between the ewe and σ_g^2 was the additive genetic variance explained by SNPs, and $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, where **I** was the identity matrix of order n = 147 and σ_e^2 was the residual variance.

Bonferroni multiple-test was used to avoid a type I error (Armstrong 2014). Bonferroni multiple-test correction was estimated at 5% genome-wide significance as 0.05/m (0.05/45,801= 1.09×10^{-6}), which corresponds to 5.96 on a $-\log_{10}(P)$ value scale. In addition, the less conservative suggestive significance threshold (Lander and Kruglyak 1995) was used, which was 1/number of SNPs, which in this case is (1/45,801 =2.18×10^{-5}), which was 4.66 on a $-\log_{10}(P)$ value scale.

Manhattan plots in which $-\log_{10}(P)$ values were plotted against their genomic locations of the markers for each trait using the qqman package in R software 4.2.1 (Turner 2014).

7.2.8 Candidate genes and functional analysis

The candidate genes were searched using Ensembl Release 112 (Martin et al. 2023), based on the *Ovis aries* (sheep) reference genome assembly ARS-UI_Ramb_v2.0 (GCA_016772045.1) (Davenport et al. 2022). Gene annotation boundaries were set at 100 Kb upstream or downstream from the position of each significantly associated intergenic SNP. The biological functions of the associated candidate genes were reviewed using the Gene Ontology (GO) tool in Ensembl.

7.3 Results

7.3.1 Descriptive statistics

Average milk production and composition, milk protein profile, and milk coagulation properties and milk pH for this flock of dairy sheep are presented in Table 7.1.

7.3.2 Manhattan plots

The Manhattan plots for milk production and composition, milk coagulation properties, and milk protein profile for dairy sheep are presented in Figure 7.1, Figure 7.2, and Figure 7.3, respectively. It is possible to observe notable clustering of SNPs for most studied traits, especially for of α_{s1}-CN, α_{s2}-CN, and α-lactalbumin in Figures 7.3 B, C, and E, respectively.

Table 7.1. Means, standard deviations (SD), minimum and maximum values, and coefficient of variation (CV) for milk production, composition, protein profile, and milk coagulation properties (MCP) of dairy sheep from a New Zealand flock.

Trait[1]	N	Mean	SD	Minimum	Maximum	CV (%)
Milk yield (L/day)	470	0.57	0.25	0.10	1.80	44
Fat (%)	470	6.32	1.26	1.87	11.24	20
Protein (%)	470	5.52	0.68	4.09	8.80	12
Lactose (%)	470	4.72	0.27	3.66	5.34	6
Casein (%)	470	4.21	0.50	3.08	6.28	12
Casein:Protein	470	0.77	0.02	0.54	0.82	3
Casein:Calcium	470	2.28	0.29	1.58	4.02	13
Calcium (mg/100 mL)	470	185.5	25.3	104.6	269.4	14
Protein profile[2] (% of total protein)						
κ-casein	470	7.28	1.30	2.60	13.80	18
α_{s1}-casein	470	27.58	1.78	20.60	32.80	6
α_{s2}-casein	470	12.78	1.58	5.90	17.80	12
β-casein	470	40.06	1.96	32.50	45.50	5
α-lactalbumin	470	1.94	0.61	0.09	4.60	31
β-lactoglobulin	470	10.36	1.25	6.20	14.30	12
MCP						
RCT (min)	451	13.3	3.2	6.86	26.0	24
K20 (min)	447	2.7	1.1	1.30	10.2	41
A30 (mm)	451	53.3	9.5	11.60	75.4	18
pH	470	6.59	0.09	6.34	6.90	1

[1]Casein:Protein= ratio of casein content to protein content; Casein:Calcium= ratio of casein content to total calcium content. [2]Protein profile: % of each protein fraction in total protein. RCT= rennet coagulation time; K20= time to reach curd firmness of 20 mm; A30= curd firmness at 30 minutes post rennet addition.

The contents of α_{s1}-CN, α_{s2}-CN, and α-LA had significant associations with SNPs at both genome-wide Bonferroni and suggestive thresholds in OAR6 (α_{s1}-CN and α_{s2}-CN), OAR20 (α_{s2}-CN), OAR25 and OAR26 (α_{s1}-CN), and OAR3 (α-LA). For α_{s2}-CN, OAR3 also had clustering but with no significant SNP associations. Despite there being a clear peak in OAR25 for κ-CN, there were no significant SNP associations for κ-CN. For β-lactoglobulin, OAR3 had the most notable clustering, but also with no significant SNP associations.

Other milk traits investigated were only significantly associated with SNPs at the suggestive threshold. These traits included milk yield, casein content, ratio of casein to protein, calcium content, ratio of casein to calcium, pH, and rennet coagulation time (RCT). A notable clustering of SNPs in OAR3 was seen for milk yield (Figure 7.1 A), with only one significant intergenic SNP over the suggestive threshold. Although no significant associations were found for fat, protein, or lactose contents (Figure 7.1 B, C, D), which was possibly due to the small dataset, there was a notable clustering of SNPs in OAR3 and OAR24 for fat, and in OAR3 and OAR15 for protein content. Two notable clusters in OAR2, and one in OAR16 were seen for lactose content.

For total casein content, a significantly associated SNP was found in OAR3, the same SNP was related to α-LA content. Several clusters across the ovine genome could be observed for ratio of casein to protein, but only one SNP over the suggestive threshold was found, in OAR2. Despite having a significant SNP for calcium content in OAR2, there was no obvious clustering of SNPs in the region, which could be due to lack of linkage disequilibrium (LD) for this trait. For ratio of casein to calcium, a significant SNP with clustering was found in OAR18, which almost reached the Bonferroni threshold.

For milk pH, a clustering with significant SNP was observed in OAR16. Additionally, the Manhattan plots evidenced clear peaks in OAR16 and OAR25 for milk coagulation properties (RCT, K20, and A30), but only one significant SNP at the suggestive threshold was found, and that was for RCT in OAR16.

The QQ-plots are presented in the Appendix 3 Figures 1-3, which showed proper correction for data stratification. In the QQ-plots of contents of α_{s1}-CN, α_{s2}-CN, and α-lactalbumin, p-values were clearly more significant than expected under the null hypothesis, with points moving towards the y-axis (Ehret 2010).

Detailed investigation into linkage disequilibrium in the breed was beyond the scope of the present study. However, it was possible to observe linkage disequilibrium in the region of the casein genes and LALBA in the linkage disequilibrium map of the SVS software (Appendix 3 Figures 4-5).

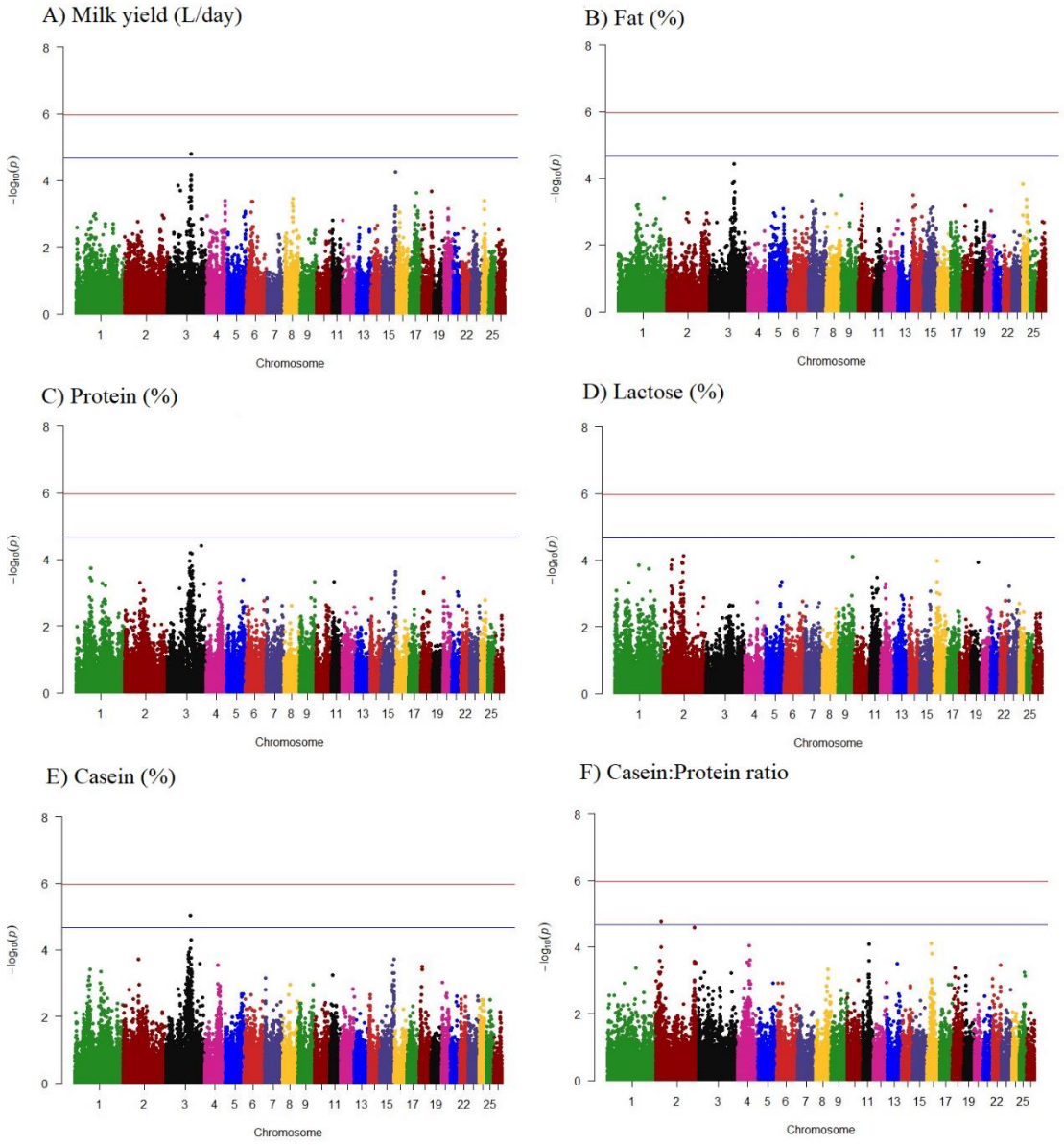


Figure 7.1. Manhattan plots for $-\log_{10}(P)$ values of marker effects for milk yield, fat, protein, lactose, and casein contents, and ratio of casein content to protein content, in A, B, C, D, E, and F, respectively. The genome-wide significance threshold of Bonferroni

correction is represented by the red line at $-\log_{10}(P)$ value = 5.96, and the suggestive significance threshold is represented by the blue line at $-\log_{10}(P)$ value = 4.66.

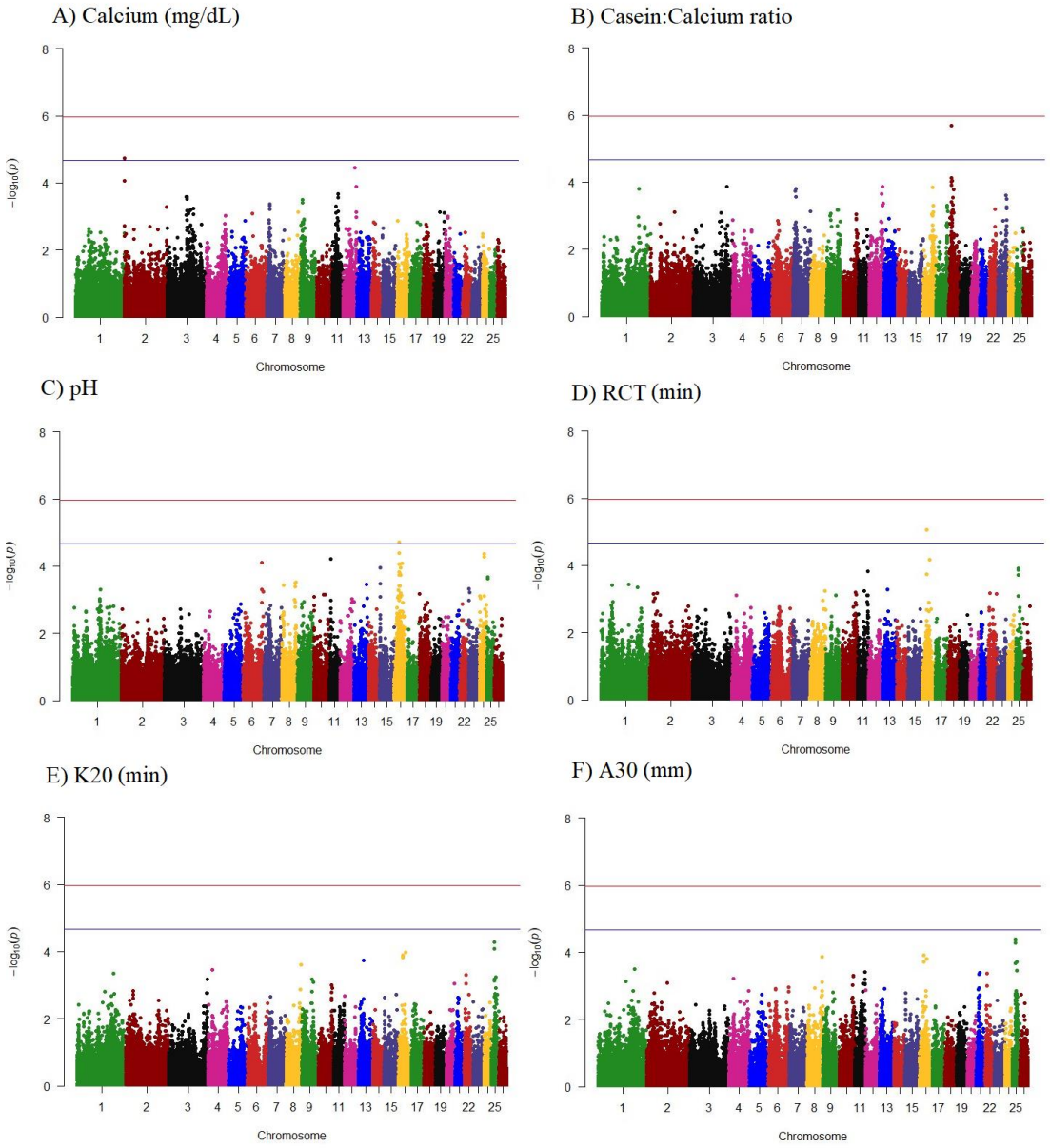


Figure 7.2. Manhattan plots for $-\log_{10}(P)$ values of marker effects for calcium content (mg/dL), ratio of casein to calcium content, pH, and milk coagulation properties (RCT, K20, and A30), in A, B, C, D, E, and F, respectively. The genome-wide significance threshold of Bonferroni correction is represented by the red line at $-\log_{10}(P)$ value = 5.96,

and the suggestive significance threshold is represented by the blue line at $-\log_{10}(P)$ value = 4.66.

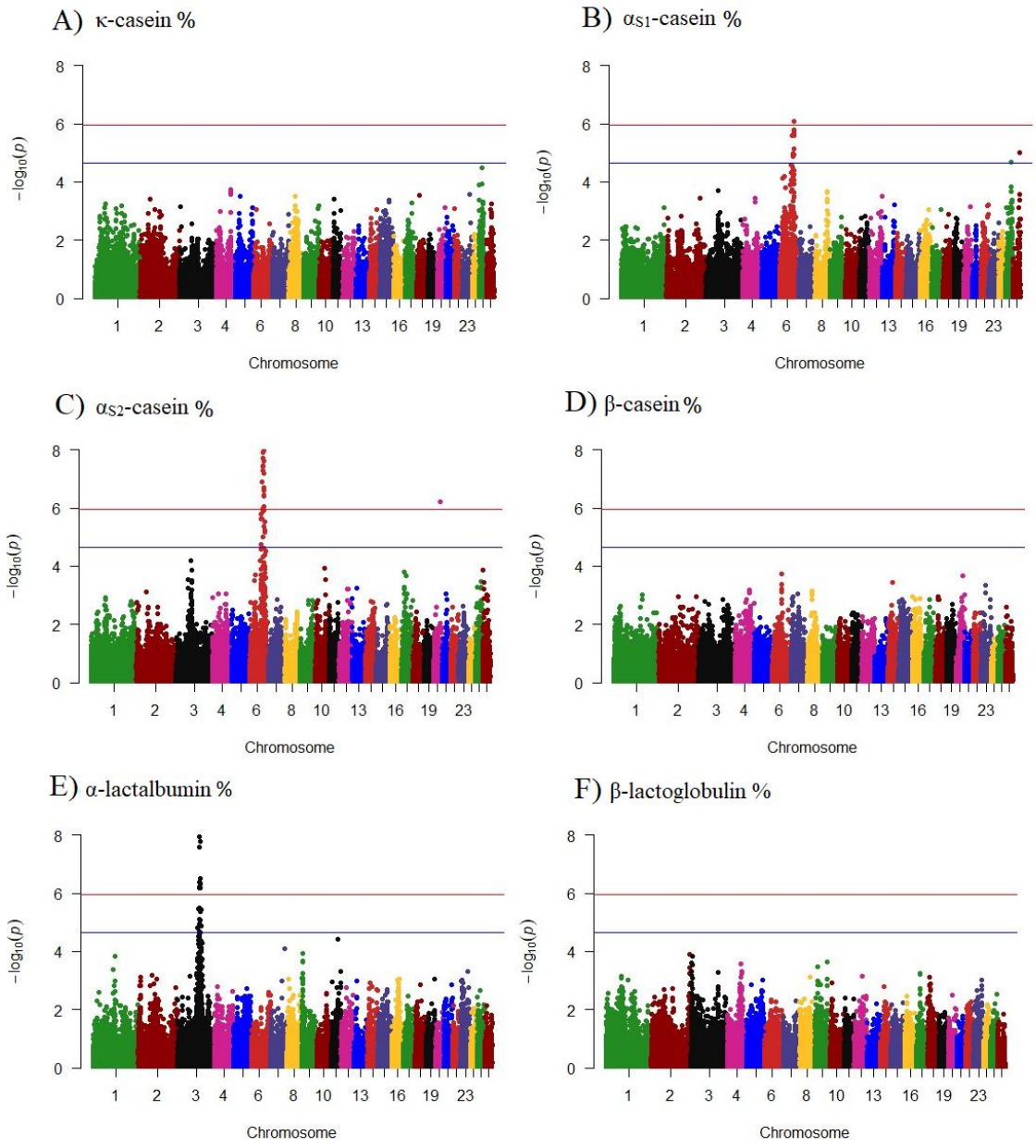


Figure 7.3. Manhattan plots for $-\log_{10}(P)$ values of marker effects for content (% in total protein of sheep milk) of κ-, α_{s1}-, α_{s2}-, β- casein, α-lactalbumin, and β-lactoglobulin, in A, B, C, D, E, and F, respectively. The genome-wide significance threshold of Bonferroni correction is represented by the red line at $-\log_{10}(P)$ value = 5.96, and the suggestive significance threshold is represented by the blue line at $-\log_{10}(P)$ value = 4.66.

7.3.3 Candidate genes and function analyses

The significant SNPs and candidate genes for milk yield, casein, ratio of casein to protein, calcium content, ratio of casein to calcium, milk pH, and RCT are presented in Table 7.2. The significant SNPs and candidate genes for contents of α_{s1}-CN, α_{s2}-CN, and α-LA, are presented in Table 7.3, Table 7.4, and Table 7.5, respectively. The full list of genes, with respective annotations and gene ontology terms are in Appendix 3 Table 1.

A total of 61 candidate genes (including novel genes) were associated with the significant SNPs. From these, 27 candidate genes were associated with intragenic SNPs. The genes BMP2K, CCDC158, CFAP299, GK2, NAA11, SCL4A4, and USO1, in OAR6, were significantly associated with both α_{s1}-CN and α_{s2}- CN contents, at the Bonferroni and/or suggestive significance levels. The gene TBK1, in OAR3, was significantly associated, at suggestive levels, with α-LA and total casein contents. The genes CNTN1 and PDZRN4, in OAR3, were associated with milk yield and with α-LA content at Bonferroni and suggestive levels. DCK was associated with both milk yield and α_{s2}- CN.

For α_{s1}-CN, a total of 12 SNPs were associated. From these, 10 SNPs were in OAR6 (87.1-96.4 Mb), being the region of 94.2-95.0 Mb with the highest number of associated SNPs. Whereas the region 96.1- 96.4 Mb had three intragenic SNPs, all in CFAP299 gene. For α_{s2}-CN, a total of 41 associated SNPs were in OAR6 (80.9-101.7 Mb). The casein genes were found to be within this region (86.2-86.4 Mb), but not within 500 Kb distance of any of the significantly associated SNPs. Two SNPs were intragenic in MAPK10.

For α-lactalbumin content, a total of 26 SNPs in OAR3 (128.5-156.6 Mb) were significantly associated. The LALBA (137.5 Mb) gene was found to be within this region, but the closest SNP to LALBA was 188.5 Kb distant. Two intragenic significant SNPs were found in ADAMTS20 and in PDZRN4, which was also associated with milk yield in the present study.

Table 7.2. The single nucleotide polymorphisms (SNPs) identified as significant at the suggestive level ($-\log_{10}$ (*P*) value ≥ 4.66 and < 5.96) for milk yield (L/day), casein (%), ratio of casein to protein (CSN:PP), calcium (mg/100 mL), ratio of casein to calcium (CSN:Ca), pH, and milk rennet coagulation time (RCT, min) for dairy sheep.

[1] Trait	SNP	Chr	Position (bp)	Ref./ MA	Ref. Freq.	Effect	SE	$-\text{Log}_{10}(P)$	Gene	SNP Annotation	Genes within 100kb
Milk yield (L/day)	OAR3_146042298.1	3	146042298	A/G	0.85	-101	23	4.80	-	Intergenic	CNTN1, PDZRN4
Casein %	OAR3_155454982.1	3	155454982	G/A	0.34	1.39	0.31	5.03	TBK1	Exon 9	
CSN:PP	OAR2_31837499.1	2	31837499	A/G	0.89	0.85	0.19	4.75	-	Intergenic	FBP1, FBP2, CTSL
Calcium (mg/100 mL)	ilmnseq_rs417754992	2	719637	G/A	0.83	-8.13	1.90	4.73	SNTG2	Intron	TPO, PXDN
CSN:Ca	OAR18_17516069.1	18	17516069	G/A	0.84	-8.43	1.77	5.67	-	Intergenic	-
pH	s69560.1	16	28915182	A/G	0.75	-0.03	0.01	4.70	-	Intergenic	EMB
RCT (min)	OAR16_23634510.1	16	23634510	A/G	0.65	-1.27	0.28	5.04	SLC38A9	Intron	DDX4, IL31RA

[1]CSN:PP= ratio of casein content to protein content; CSN:Ca= ratio of casein content to calcium content; RCT= rennet coagulation time (min).

Table 7.3. The single nucleotide polymorphisms (SNPs) identified as significant at the Bonferroni level ($-\log_{10}$ (P) value ≥ 5.96) and at the suggestive level ($-\log_{10}$ (p) value ≥ 4.66 and < 5.96) level for **α_{s1}-casein** (% in total protein) in dairy sheep milk.

SNP	Chr	Position (bp)	Ref./ MA	Ref. Freq.	Effect	SE	$-\text{Log}_{10}(P)$	Gene	SNP Annotation	Genes within 100kb	Significance level
ilmnseq_rs413585365	6	87126636	A/G	0.84	-1.03	0.22	5.58	-	Intergenic	SLC4A4, DCK	Suggestive
OAR6_91381996.1	6	91381996	A/G	0.85	-0.98	0.22	4.86	USO1	Intron		Suggestive
OAR6_91995865.1	6	91995865	A/G	0.81	-0.92	0.21	4.97	CCDC158	Intron		Suggestive
s43490.1	6	94276957	G/A	0.83	-1.07	0.21	6.09	BMP2K	Intron		Bonferroni
OAR6_94793686.1	6	94793686	G/A	0.82	-0.97	0.22	5.15	-	Intergenic	GK2, NAA11	Suggestive
s59224.1	6	94902165	A/G	0.82	-1.05	0.22	5.80	-	Intergenic	GK2	Suggestive
OAR6_94954855.1	6	94954855	G/A	0.83	-0.96	0.22	4.94	-	Intergenic	-	Suggestive
OAR6_96130389.1	6	96130389	A/G	0.84	-1.03	0.22	5.58	CFAP299	Intron		Suggestive
OAR6_96189267.1	6	96189267	A/G	0.84	-1.03	0.22	5.58	CFAP299	Intron		Suggestive
OAR6_96431721.1	6	96431721	A/C	0.84	-1.05	0.22	5.71	CFAP299	Intron		Suggestive
OAR25_40232454.1	25	40232454	A/G	0.44	0.64	0.15	4.69	WAPL	Intron		Suggestive
s02584.1	26	46615889	G/A	0.88	1.15	0.26	5.00	-	Intergenic	-	Suggestive

Table 7.4. The single nucleotide polymorphisms (SNPs) identified as significant at the Bonferroni level ($-\log_{10}$ (*P*) value ≥ 5.96) and at the suggestive level ($-\log_{10}$ (*p-value*) ≥ 4.66 and < 5.96) level for **α_{s2}-casein** (% in total protein) in dairy sheep milk.

Lead SNP	Chr	Position (bp)	No. SNPs	Ref./ MA	Ref. Freq.	Effect	SE	-Log_{10}(*P*)	Gene	SNP Annotation	Genes within 100kb	Significance level
OAR6_80961711.1	6	80961711	1	G/A	0.88	1.13	0.23	5.78	-	Intergenic	ENSOARG00020024401.2	Suggestive
OAR6_81183719.1	6	81183719	1	G/A	0.91	1.09	0.25	4.76	-	Intergenic	ENSOARG00020024401.2	Suggestive
OAR6_81434797.1	6	81434797	1	A/C	0.1	-1.19	0.25	5.63	-	Intergenic		Suggestive
s39455.1	6	81609420	1	A/G	0.9	1.19	0.25	5.63	EPHA5	Intron		Suggestive
ilmnseq_rs425296828	6	84420871	1	A/G	0.8	1.00	0.19	6.89	TMPRSS11D	Intron		Bonferroni
ilmnseq_rs413585365	6	87126636	1	A/G	0.83	1.19	0.2	8.22	-	Intergenic	SLC4A4, DCK	Bonferroni
OAR6_87592155.1	6	87592155	1	G/A	0.81	1.14	0.2	8.25	-	Intergenic	SLC4A4, GC	Bonferroni
OAR6_88110298.1	6	88110298	1	C/A	0.8	0.92	0.19	5.98	-	Intergenic	NPFFR2, ADAMTS3	Bonferroni
OAR6_88303825.1	6	88303825	1	A/G	0.8	0.92	0.19	5.98	ADAMTS3	Intron		Bonferroni
OAR6_88678679.1	6	88678679	1	A/G	0.8	0.92	0.19	5.98	-	Intergenic	ENSOARG00020003468	Bonferroni
OAR6_91381996.1	6	91381996	1	A/G	0.84	1.23	0.21	8.47	USO1	Intron		Bonferroni
OAR6_91640306.1	6	91640306	1	A/G	0.85	1.24	0.21	8.31	CXCL10	Intron		Bonferroni
OAR6_91765793.1	6	91765793	1	A/G	0.83	0.96	0.2	5.89	NUP54	Intron		Suggestive
OAR6_91995865.1	6	91995865	1	A/G	0.81	1.22	0.19	9.41	CCDC158	Intron		Bonferroni
OAR6_92241864.1	6	92241864-92321965	2	A/G	0.79	1.06	0.19	7.73	SHROOM3	Intron		Bonferroni
OAR6_92730863.1	6	92730863	1	G/A	0.83	1.08	0.2	7.44	-	Intergenic	CCNI, CCNG2	Bonferroni
ilmnseq_rs405099401	6	92837990	1	G/A	0.81	1.03	0.19	7.28	-	Intergenic	CCNG2	Bonferroni

OAR6_93031365.1	6	93031365	1	G/A	0.79	1.08	0.19	8.19	-	Intergenic	CXCL13	Bonferroni
ilmnseq_rs409838119	6	93370144	1	A/T	0.85	0.92	0.21	4.99	-	Intergenic		Suggestive
OAR6_93397312.1	6	93397312	1	G/A	0.83	1.11	0.19	7.91	-	Intergenic	MRPL1, FRAS1	Bonferroni
s43490.1	6	94276957	1	G/A	0.83	1.33	0.2	10.37	BMP2K	Intron		Bonferroni
OAR6_94793686.1	6	94793686	1	G/A	0.82	1.11	0.2	7.61	-	Intergenic	NAA11, GK2	Bonferroni
s59224.1	6	94902165	1	A/G	0.82	1.09	0.2	7.20	-	Intergenic	GK2	Bonferroni
OAR6_94954855.1	6	94954855	1	G/A	0.83	1.16	0.2	7.98	-	Intergenic		Bonferroni
OAR6_95349948.1	6	95349948	1	A/G	0.82	0.89	0.19	5.37	ANTXR2	Intron		Suggestive
OAR6_96130389.1	6	96130389-96431721	3	A/G	0.83	1.19	0.2	8.22	CFAP299	Intron		Bonferroni
OAR6_97085567.1	6	97085567-97185219	2	A/G	0.79	0.97	0.2	6.05	RASGEF1B	Intron		Bonferroni
OAR6_97397109_X.1	6	97397110	1	A/G	0.79	0.96	0.2	5.94	RASGEF1B	Intron		Suggestive
s52112.1	6	97682782	1	G/A	0.82	1.01	0.2	6.39	-	Intergenic	RASGEF1B	Bonferroni
OAR6_97927928.1	6	97927928	1	A/G	0.82	1.02	0.2	6.43	HNRNPDL	Exon 1		Bonferroni
s71640.1	6	98157876	1	A/G	0.81	1.02	0.2	6.60	-	Intergenic	TMEM150C, SCD5, ENSOARG00020035089	Bonferroni
OAR6_98214895.1	6	98214895	1	A/G	0.82	1.04	0.2	6.71	SCD5	Intron		Bonferroni
OAR6_99384589.1	6	99384589	1	G/A	0.8	0.92	0.19	5.92	-	Intergenic	-	Suggestive
OAR6_99947976.1	6	99947976	1	A/G	0.81	0.99	0.19	6.70	-	Intergenic	CDS1, NKX6-1	Bonferroni

OAR6_101467729.1	6	101467729	1	A/C	0.55	0.69	0.15	5.54	ARHGAP24	Intron	LD with MAPK10	Suggestive
OAR6_101563461.1	6	101563461	1	A/G	0.46	-0.67	0.15	5.24	MAPK10	Intron	ARHGAP24	Suggestive
s42066.1	6	101746181	1	G/A	0.79	0.83	0.18	5.17	MAPK10	Intron	-	Suggestive
ilmnseq_rs421607124	20	45934168	1	G/C	0.84	0.98	0.19	6.20	LOC105605956	Intron	-	Bonferroni

Table 7.5. The single nucleotide polymorphisms (SNPs) identified as significant at the Bonferroni level ($-\log_{10}$ (*p-value*) ≥ 5.96) and at the suggestive level ($-\log_{10}$ (*p-value*) ≥ 4.66 and < 5.96) level for **α-lactalbumin** (% in total protein) in dairy sheep milk.

Lead SNP	Chr	Position (bp)	No. SNPs	Ref./MA	Ref. Freq.	Effect	SE	-Log$_{10}$(*p*)	Gene	SNP Annotation	Genes within 100kb	Significance level
OAR3_128546505.1	3	128546505	1	G/A	0.92	-0.33	0.08	4.81	-	Intergenic	-	Suggestive
ilmnseq_rs403176291	3	137390760	1	G/A	0.45	-0.18	0.04	5.47	-	Intergenic	FKBP, CCDC65, RND1, CACNB3, ADCY6, SPMIP11, CCNT1	Suggestive
s53492.1	3	143343824	1	G/A	0.36	-0.18	0.04	5.45	ADAMTS20	Intron	-	Suggestive
s31828.1	3	143359947	1	G/A	0.38	-0.20	0.04	6.37	ADAMTS20	Intron	-	Bonferroni
s50395.1	3	143618027	1	C/A	0.68	-0.19	0.05	4.69	-	Intergenic	-	Suggestive
OAR3_143745966.1	3	143745966	1	G/A	0.79	-0.22	0.05	5.09	-	Intergenic	-	Suggestive
OAR3_145276010.1	3	145276010	1	C/A	0.79	-0.28	0.05	7.57	-	Intergenic	-	Bonferroni
OAR3_145295705.1	3	145295705	1	G/A	0.66	-0.24	0.04	7.93	-	Intergenic	-	Bonferroni
OAR3_145344922.1	3	145344922	1	G/A	0.57	-0.19	0.04	4.96	-	Intergenic	-	Suggestive
s25992.1	3	145632982	1	A/C	0.7	-0.2	0.04	5.5	PDZRN4	Intron	-	Suggestive
s34624.1	3	145835332-145931284	2	A/G	0.34	-0.19	0.04	6.22	PDZRN4	Intron	-	Bonferroni
OAR3_146042298.1	3	146042298	1	A/G	0.85	-0.35	0.06	8.71	-	-	CNTN1, PDZRN4	Bonferroni
s35014.1	3	146168927-146281949	4	C/A	0.71	-0.26	0.05	7.77	CNTN1	Intron	-	Bonferroni and Suggestive

OAR3_146519457.1	3	146519457	1	A/G	0.59	-0.19	0.04	5.37	-	Intergenic	ENSOARG00020036542.1	Suggestive
OAR3_146751517.1	3	146751517	1	G/A	0.76	-0.24	0.05	6.31	MUC19	Intron		Bonferroni
OAR3_147028849.1	3	147028849	1	A/C	0.82	-0.34	0.05	9.5	-	Intergenic	LRRK2, SLC2A13	Bonferroni
OAR3_148569270.1	3	148569270	1	C/A	0.77	-0.24	0.05	6.33	CPNE8	Intron	-	Bonferroni
OAR3_151463131.1	3	151463131	1	A/G	0.72	-0.21	0.05	5.06	-	-	-	Suggestive
OAR3_155081787.1	3	155081787	1	A/G	0.58	-0.19	0.04	4.86	-	-	TBC1D30	Suggestive
OAR3_155454982.1	3	155454982	1	G/A	0.34	-0.18	0.04	5.42	TBK1	Exon 9	-	Suggestive
OAR3_156007768.1	3	156007768	1	G/A	0.29	-0.19	0.04	4.87	SRGAP1	Intron	-	Suggestive
OAR3_156555651.1	3	156555651	1	G/A	0.83	-0.24	0.05	4.87	-	Intergenic	AVPR1A	Suggestive

7.4 Discussion

Average production level of this flock in the studied season has been previously discussed (Marshall et al. 2023, Chapter 3), and the relatively low milk production was attributed primarily to limited pasture availability, also peak production in early lactation was missed due to the lamb suckling period. The milk coagulation properties during the once-a-day milking period have also been discussed (Marshall et al. 2024c, Chapter 4), and they were within the range previously reported for dairy sheep. The effect of protein polymorphisms on milk composition, coagulation properties, and protein profile in dairy sheep from this flock was investigated in our previous study (Marshall et al. 2025, Chapter 5).

7.4.1 Manhattan plots for milk production and composition

In agreement with others (Moioli et al. 2015; Garcia-Gamez et al. 2012), OAR3 was of notable importance for milk production and composition traits. In our study, the region between 145 and 146 Mb was important for milk yield, and the region between 151 and 156 Mb of OAR3 were relevant for fat and protein percentages. This partly agrees with the findings of Moioli et al. (2015) as the region 145 Mb was significantly associated but with protein content. For Gacia-Gamez et al. (2012), OAR3 was also important for fat and protein percentages but in position 137.3 Mb. For Carta et al. (2014) position 131.31 Mb of OAR3 was associated with fat and protein yields, and position 141.53 Mb with protein content.

The wide region found in the present study for α-LA content in OAR3 (128.5-156.6 Mb) includes the QTL previously reported by Gutiérrez-Gil et al. (2009) for protein content (131.74-134.46 Mb). However, one of our significant SNPs was not as distant from LALBA as the distance reported by Gutiérrez-Gil et al. (2009) (188.5 kb vs 2.7 Mb respectively). But Garcia-Gamez found that a marker on OAR3 that showed the highest significant association with protein percentage was located at the third intron of LALBA.

Garcia-Gamez (2012) found the region between 29.1 and 29.6 Mb, and position 23.7 Mb of OAR20 with significant associations for protein and fat percentages. Whereas in our study position OAR20 was associated with α_{s2}-casein content, at position 45Mb.

For lactose percentage, OAR2 between 122-126 Mb was important in our study. Interestingly, for Garcia-Gamez, OAR2 was important for yield traits (milk, fat, and

lactose yields), but concentrated between 42.7 and 63.2 Mb, and between 53.0 and 58.0 Mb. The focus below will be on candidate genes associated with the traits that showed the most significant SNP signals.

7.4.2 Candidate genes for milk production and composition

In agreement with the present study, the gene PDZRN4 has also been recently associated with milk yield in New Zealand dairy sheep by another research group (Costilla et al. 2023) using three large flocks distinct from this one, evidencing that this gene's function should be further investigated in New Zealand dairy sheep. PDZRN4 is involved in protein degradation pathways. In other livestock species, this gene has also been associated with heat stress of dairy cows, which can cause a decrease in the expression of genes involved in milk production (Czech et al. 2023), and with intramuscular fat content in pigs (Wang et al. 2021).

The biological processes of gene CNTN1, associated with milk yield and α-lactalbumin, include positive regulation of sodium transport, gene expression, protein and carbohydrate binding. Both candidate genes associated with milk yield (CNTN1 and PDZRN4) have also been previously correlated with milk production in Holstein dairy cattle (Shin et al. 2014), and CNTN1 has also been associated with udder and teat scores in Angus cattle (Devani et al. 2020). CNTN1 gene codes for Contactin 1, a protein involved in cell adhesion. This gene has been associated with myopathy and muscular weakness (Compton et al. 2008). It is likely that this gene is associated with milk production in dairy animals due to the dependence of udder structure over repeated parities on muscular vigour, indeed our previous study reported the antagonistic phenotypic correlation between milk production and udder conformation (Marshall et al. 2024a, Supplementary information).

The gene TBK1, a candidate gene (SNP in Exon 9) for casein content and α-LA, is involved in immune response and in the induction of IFN-β production in goats and cows, which was confirmed by the over-expression of the full-length TBK1 gene in a viral infection experiment (Yang et al. 2022). Also, TBK1 was considered a pregnancy-specific protein involved in control of bacterial infection and viral immunity (Han et al. 2008). While TBK1 is not directly associated with milk casein or α-LA, they could be indirectly related through regulation of gene expression and immune response. The gene CTSL may

affect the ratio of casein to total protein as its activity could lead to the degradation of caseins or other milk proteins, affecting their relative proportions.

The candidate gene for calcium content, SNTG2, has been associated with osteoporosis in women (Jales Neto et al. 2020). One of its biological processes is structure molecule activity and SNTG2 is highly expressed in the musculoskeletal system (Moon et al. 2017). It is possible that variations on SNTG2 might influence calcium metabolism in the organism. However, in the literature, other genes were found to be associated with Ca content in bovine milk, such as SLC37A1 in *Bos taurus* autosome BTA 1 and ANKH in BTA 16 (Sanchez et al. 2021), the biological processes of these genes are related to ion transmembrane transport.

The functions of the candidate gene for milk pH, EMB, includes cell adhesion, which could make sense as milk pH is likely to be influenced by the influx of minerals when the junction between mammary epithelial cells is compromised either in cases of infection (mastitis) or of mechanical tissue damage in late lactation (Hettinga 2019). Embigin's role in cell adhesion could affect the integrity of mammary epithelial cells and affect the balance of ions and pH in milk. In addition, the gene EMB has been previously associated with milk yield in dairy sheep (Li et al. 2020).

7.4.3 Candidate genes for contents of α_{s1}- and α_{s2}-casein

Although it is widely known that variations in CSN1S1 and CSN1S2 genes are responsible for phenotypic variants of α_{s1}-CN and α_{s2}-CN proteins (De Pascale et al. 2022), and are associated with differences in the quantity of these protein fractions (Pirisi et al. 1999; Huang et al. 2012), no significant SNP associations in the casein genes were found due to the bead chip coverage, and further investigation on variation of casein genes with genomic imputation could not be done. However, there was a linkage between DCK, SLC4A4, and GC and the region involving the casein cluster. Overall, most genes associated here with protein profile have also been reported by Marina et al. (2020) in the enrichment and association analysis of genes associated with milk and cheese-making traits.

The genes BMP2K, CCDC158, CFAP299, GK2, NAA11, SCL4A4, DCK and USO1 were candidates for both α_{s1}-CN and α_{s2}-CN. The most significant SNP for α_{s1}-CN, $-\log_{10}(P)$ =

6.09, and α_{s2}-CN, $-\log_{10}(P) = 10.37$, was in gene BMP2K which is involved in protein phosphorylation. This finding is logical because the level of phosphorylation is related to the concentration of α_{s1}-CN and α_{s2}-CN. This finding could be of great importance for the technological and functional properties of sheep milk (Bijl et al. 2020). This gene's indirect role in phosphorylation of milk caseins should be further investigated.

Other genes involved in phosphorylation processes and kinases in the present study included BMP2K, CCNT1, EPHA5, FBP1, GK2, LRRK2, MAPK10, NPFFR2, and TBK1. Attention should be given to the potential roles these genes may play on casein phosphorylation, which is catalysed by protein kinases that attach phosphate groups to specific AA residues Ser and Thr (Mercier 1981), after the synthesis of the polypeptide chains in the Golgi apparatus of the mammary epithelial cell (Bingham and Farell Jr 1977). In addition, the results from Moioli et al. (2015) showed that the most represented categories of genes associated with protein content had functions related to the phosphorus/phosphate metabolic processes.

Further investigation in this topic with the quantification of phosphorylation level of sheep milk caseins could be done using LC/ESI-MS methods. In dairy cows, milk protein profile has been associated with several regions across 20 chromosomes, especially the regions coding for protein variants of β-CN, κ-CN, or β-LG, and DGAT1 (Schopen et al. 2011). In addition, it has been found that the phosphorylation level of isoforms of α_{s1}-CN were associated with different genes (DGAT1 vs β-LG), which suggested that phosphorylation of isoforms is mediated by different enzymes (Bijl et al. 2014). Furthermore, up- and down-regulatory systems were proposed for phosphorylation levels of α_s-CNs (Fang et al. 2016).

In addition, of notable importance for α_{s1}-CN and α_{s2}-CN were the genes DCK, SLC4A4, and GC, which are proximate to the casein cluster and were found to be in linkage disequilibrium with the casein region, especially DCK and SLC4A4, as mentioned previously. In agreement, the gene SLC4A4 has been previously associated with α_{s2}-CN content in dairy cows (Dadousis et al. 2017b). This gene has also been associated with other milk traits in dairy cattle, including milk yield (Jiang et al. 2019; Kim et al. 2021; Pedrosa et al. 2021), protein yield (Jiang et al. 2019), fat yield and fat percentage (Pedrosa et al. 2021), mastitis (Sodeland et al. 2011; Wu et al. 2015), and with milk curd-firming characteristics (Bertelsen et al. 2016; Dadousis et al. 2017a).

The SLC4A4 is involved in the regulation of intracellular pH, and secretion and absorption of bicarbonate (Dadousis et al. 2017b), and active transport of glucose which is uptaken by the mammary epithelial cells for milk synthesis (Pedrosa et al. 2021). The bicarbonate transport facilitated by SLC4A4 is also linked to calcium ion concentration and homeostasis (Thornell and Bevensee 2015). Since α_s-CNs interact with calcium via phosphoserine and phosphothreonine, any changes in calcium availability could influence the micellization behaviour and physicochemical properties of caseins in milk.

Despite the genes DCK and GC not being previously associated with milk protein profile, they have also been considered top candidate genes for milk yield (Pedrosa et al. 2021) and mastitis in dairy cows (Sodeland et al. 2011; Wu et al. 2015), and GC has been associated with fertility traits (Jiang et al. 2019). GC is involved in calcium homeostasis and vitamin D transport.

Also, no previous association was found between milk protein profile and ADAMTS3 and NPFFR2, but these genes were associated with milk yield and protein yield in a large GWAS study with Holstein dairy cattle in the USA (Jiang et al. 2019), and ADAMTS3 with milk yield of dairy goats in China (Ni et al. 2024). It was also found that the expression of ADAMTS3 significantly increased during inflammatory response in bovine mammary epithelial cells (Sheng et al. 2023). Its roles in extracellular matrix remodelling, cell signalling, proteolytic activity, and tissue remodelling suggest that it could influence the mammary gland environment in ways that impact protein secretion.

The gene NPFFR2 was previously considered a strong candidate gene affecting mastitis occurrence in cattle (Sahana et al. 2014; Wu et al. 2015; Zhang et al. 2016). NPFFR2 has been suggested to regulate feeding behaviour and energy expenditure in mammals and has been linked to heat tolerance (Cheruiyot et al. 2021), so it could indirectly affect the mammary gland's ability to synthesize and secrete milk proteins.

The gene USO1, associated with both α_{s1}-CN, $-\log_{10}(P) = 4.86$, and α_{s2}-CN, $-\log_{10}(P) = 8.47$) is involved in protein amino acid binding and one of its processes is intracellular protein transport. It has been associated with milk yield in buffaloes (El-Halawany et al. 2017). The gene CXCL13 was one of the important candidate genes for α_{s2}-CN only, $-\log_{10}(P)= 8.19$, and has been associated with milk traits and lactation persistency (Pedrosa et al. 2021). The CXC genes code for chemokines which are important in inflammatory response and have been associated with clinical mastitis (Sodeland et al. 2011). The gene

HNRNPDL's only function discovered was nucleic acid binding. No biological process or molecular function were found for CFAP299 or CCDC158.

7.4.4 Candidate genes for the content of α-lactalbumin

For α-lactalbumin, OAR3 was important, the candidate gene CCNT1 was within 80kb distance from LALBA, and a small LD was observed in the region (detailed in Appendix 3 Figure 5). The CCNT1's biological process is cyclin-dependent protein serine/threonine kinase regulator activity. Interestingly, CCNT1 has been previously associated with protein percentage in Assaf and Churra breeds (Marina et al. 2021).

Some of the relevant genes for α-LA, CNTN1, PDZRN4, and TBK1, were previously discussed in the milk production and composition section. It is important to highlight that α-LA is the regulatory protein of the lactose synthase enzyme system that catalyses and regulates the synthesis of lactose in the lactating mammary gland, which increases the volume of secreted fluids through osmotic effects and serves as a primer for the synthesis of oligosaccharides (Brew 2012), therefore it is logical to have common genes between α-LA and milk production traits.

The other strong candidate gene for α-LA, ADAMTS20, is involved in procollagen processing, extracellular matrix remodelling, inflammation, cell migration and angiogenesis (Kelwick et al. 2015), mammary cell differentiation, lactogenic activity of mammary epithelial cells, and stimulation of synthesis of milk proteins (Riley et al. 2010; Kozlowski et al. 2011). Interestingly, ADAMTS20 has been associated with coat colour in cattle (Drögemüller et al. 2009), with milk traits in dairy goats (Kang et al. 2020), and other ADAMTS genes have been associated with mastitis in cattle (Sheng et al. 2023).

A further candidate gene for α-LA, MUC19 (mucin protein), has been correlated with growth traits of Chinese cattle (Chen et al. 2023), and considered as artificial selection signature for milk production in a Brazilian local cattle breed (Campos et al. 2017). Mucins are the main component of mucus, a glycosylated protein secreted by epithelial cells (Belley et al. 1996). Interestingly, the candidate genes for α-LA, CNTN1, LRRK2, and CPNE8 were previously associated with udder scores in Canadian Angus cows (Devani et al. 2021).

7.4.5 Manhattan plots and candidate genes for MCP and pH

For RCT and milk pH, OAR16 was important, with significantly associated SNPs. Clustering of SNPs in OAR16 was also evidenced in the Manhattan plots for lactose, ratio of casein-to-protein, and ratio of casein-to-calcium, K20, and A30, despite the SNPs not being significant. This indicates that the genetic control of MCP, pH, lactose, ratio of casein-to-protein, and ratio of casein-to-calcium is likely to be related. In GWAS interpretation, it is important to consider not only single SNP information but also the "hot" zones within the animal genome, the QTLs where interactions among coding and non-coding regions can strongly influence the overall gene expression (Caroli et al. 2009).

Although the PRL gene was not a candidate in the present study, this gene is also within OAR16 (38.969–39.028 Mb) but over 200 kb distant from the candidate genes EMB (for pH) and SLC38A9 (for RCT). Others have reported a SNP in PRL to have an effect on MCP in Sarda sheep (Dettori et al. 2020), which was suggested to be due to the negative effect of some isoforms of PRL on the activation of milk protein gene transcription (Cassy et al. 1998).

In addition, OAR25 was important for RCT, K20, and A30, with notable clustering of SNPs, which was also evidenced for κ-CN, despite the SNPs not being significant, and for α_{s1}-CN with a significant SNP in candidate gene WAPL. This indicates that MCP could also be related to the genetic control of κ-CN and α_{s1}-CN. Further investigation into genomic correlations between RCT (the MCP with the strongest SNP signal) and the other milk traits (see Appendix 3 Table 2 and Table 3), evidenced the strong correlations with ratio of casein to calcium (r_G= 0.92, SE= 0.25), pH (r_G= 0.88, SE= 0.13), lactose (r_G= -0.86, SE= 0.22), and ratio of casein to protein (r_G= -0.77, SE= 0.15), which support our previous study on pedigree-based genetic correlations (Marshall et al. 2024b).

Surprisingly, neither OAR3, where LALBA and BLG are located, nor OAR6, where the casein genes are located, were found to be important hot zones for MCPs in the present study. Gaspa et al. (2016) also did not find significant associations with coagulation properties in OAR6, but in OAR12. In contrast, Bertelsen et al. (2016) found that a SNP close to SLC4A4 (BTA 6) had an additive genetic effect on curd firming rate in bovine milk, and the association was suggested to be due to LD with the casein gene cluster.

For non-coagulating milk, Gaspa et al. (2022a) reported genes not reported here, those genes were mostly linked to mammary gland metabolism, udder health status, and milk

compound known also to affect the ability of milk to coagulate. In partial agreement with our trends, Marina et al. (2020) also found OAR25, region 9.56 Mb, associated with A30, but in our study was region 21.54 Mb.

It has also been suggested that different GWAS findings between studies evidence that the different selection strategies of different breeds or populations largely determine quality of milk for processing (Caballero-Villalobos et al. 2018).

7.5 Conclusion

The present GWAS study on milk production, composition, and milk coagulation traits of dairy sheep found candidate genes previously associated to milk production and composition, mastitis, immune response, and udder conformation in dairy farm animals. These genes are likely to be indirectly influencing the studied milk traits and their exact mechanisms could be further investigated. Several genes reported here are involved in phosphorylation processes and kinases. The gene PDZRN4 seems to play a significant role in milk production in New Zealand dairy sheep, and the gene SLC4A4 could be playing a role in α_{s2}-CN content.

Another finding of this study is the distinct genetic background for protein profile and milk coagulation properties. The genetic background for milk coagulation properties was found to be more similar to that of the ratio of casein to calcium, pH, lactose, and the ratio of casein to protein, rather than being solely attributed to casein genes. This suggests that MCPs can be influenced by factors other than milk protein variants.

The quantity of casein fractions in total protein was related to genes close to the casein gene cluster as well as genes involved in the phosphorylation process. Little is known about the genes regulating the phosphorylation process in both bovine and ovine milks and could be an area of further investigation in dairy sheep. To the best of our knowledge, this study represents the first genome-wide association study (GWAS) focused on dissecting the genetic basis of protein composition in dairy sheep, specifically examining the quantities of individual casein and whey protein fractions.

The findings of the present study lack robustness for large-scale applications due to the small sample size. Therefore, further genomic studies must be performed with larger populations of dairy sheep. Studies on gene expression could also validate the present results. It is recommended that, beyond GWAS, linkage disequilibrium is investigated to

search for QTLs for marker-assisted selection. Whole genome sequencing could help identify the exact causative DNA polymorphisms, which could be used for gene-assisted selection in dairy sheep.

References

Amalfitano N, Mota LFM, Rosa GM, Cecchinato A, Bittante G. 2022. Role of CSN2, CSN3, and BLG genes and the polygenic background in the cattle milk protein profile. Journal of Dairy Science. 105: 6001-6020.

Ariyarathne HBPC, Correa-Luna M, Blair HT, Garrick DJ, Lopez-Villalobos N. 2021. Identification of genomic regions associated with concentrations of milk fat, protein, urea and efficiency of crude protein utilization in grazing dairy cows. Genes. 12: 456.

Armstrong RA. 2014. When to use the B onferroni correction. Ophthalmic and Physiological Optics. 34: 502-508.

Belley A, Keller K, Grove J, Chadee K. 1996. Interaction of LS174T human colon cancer cell mucins with Entamoeba histolytica: an in vitro model for colonic disease. Gastroenterology. 111: 1484-1492.

Bertelsen H, Gregersen V, Poulsen N, Nielsen RO, Das A, Madsen LB, Buitenhuis A, Holm L-E, Panitz F, Larsen LB. 2016. Detection of genetic variation affecting milk coagulation properties in Danish Holstein dairy cattle by analyses of pooled whole-genome sequences from phenotypically extreme samples (pool-seq). Journal of Animal Science. 94: 1365-1376.

Bijl E, Holland JW, Boland M. 2020. Posttranslational modifications of caseins. Milk proteins, Elsevier. Pp. 173-211.

Bijl E, van Valenberg H, Huppertz T, van Hooijdonk A, Bovenhuis H. 2014. Phosphorylation of α_{s1}-casein is regulated by different genes. Journal of Dairy Science. 97: 7240-7246.

Bingham EW, Farell Jr HM. 1977. Phosphorylation of casein by the lactating mammary gland: a review. Journal of Dairy Science. 60: 1199-1207.

Bobe G, Beitz D, Freeman A, Lindberg G. 1999. Effect of milk protein genotypes on milk protein composition and its genetic parameter estimates. Journal of Dairy Science. 82: 2797-2804.

Bobe G, Beitz DC, Freeman AE, Lindberg GL. 1998. Separation and quantification of bovine milk proteins by reversed-phase high-performance liquid chromatography. Journal of Agricultural and Food Chemistry. 46: 458-463.

Bonfatti V, Grigoletto L, Cecchinato A, Gallo L, Carnier P. 2008. Validation of a new reversed-phase high-performance liquid chromatography method for separation and quantification of bovine milk protein genetic variants. Journal of Chromatography A. 1195: 101-106.

Bramanti E, Sortino C, Onor M, Beni F, Raspi G. 2003. Separation and determination of denatured α_{s1}-, α_{s2}-, β-and κ-caseins by hydrophobic interaction chromatography in cows', ewes' and goats' milk, milk mixtures and cheeses. Journal of Chromatography A. 994: 59-74.

Brew K. 2012. α-Lactalbumin. Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects, 4th Edition, Springer. Pp. 261-273.

Caballero-Villalobos J, Figueroa A, Xibrraku K, Angón E, Perea J, Garzón A. 2018. Multivariate analysis of the milk coagulation process in ovine breeds from Spain. Journal of Dairy Science. 101: 10733-10742.

Campos BM, do Carmo AS, da Silva TBR, Verardo LL, de Simoni Gouveia JJ, Malhado CHM, da Silva MVGB, Carneiro PLS. 2017. Identification of artificial selection signatures in Caracu breed lines selected for milk production and meat production. Livestock Science. 206: 82-87.

Caroli A, Chessa S, Erhardt G. 2009. Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. Journal of Dairy Science. 92: 5335-5352.

Carta A, Usai MG, Sechi T, Miari S, Sechi S, Salaris SL, Mulas G, Barillet F, Elsen JM, Casu S. 2014. Exploring the genetic variation between Sarda and Lacaune dairy sheep breeds by genome wide association study on economic traits. Proceedings, 10th Word Congress of Genetics Applied to Livestock Production, Paper 339.

Cassy S, Charlier M, Belair L, Guillomot M, Charron G, Bloch B, Djiane J. 1998. Developmental expression and localization of the prolactin receptor (PRL-R) gene in ewe mammary gland during pregnancy and lactation: estimation of the ratio of the two forms of PRL-R messenger ribonucleic acid. Biology of Reproduction. 58: 1290-1296.

Chen Y, Peng W, Zhang Z, Liu X, Yang P, Fu C, Zhang J, Wang H, Zhou S, Lei C. 2023. The relationship between MUC19 copy number variation and growth traits of Chinese cattle. Gene. 851: 147010.

Cheruiyot EK, Haile-Mariam M, Cocks BG, MacLeod IM, Xiang R, Pryce JE. 2021. New loci and neuronal pathways for resilience to heat stress in cattle. Scientific Reports. 11: 16619.

Compton AG, Albrecht DE, Seto JT, Cooper ST, Ilkovski B, Jones KJ, Challis D, Mowat D, Ranscht B, Bahlo M. 2008. Mutations in contactin-1, a neural adhesion and neuromuscular junction protein, cause a familial form of lethal congenital myopathy. The American Journal of Human Genetics. 83: 714-724.

Costilla R, Chan P, McRae K, McEwan J, Dodds K, Newman S-A, Baird H, Henry H, Brauning R, Cullen N. 2023. Genome-wide association studies for milk traits in New Zealand dairy sheep. New Zealand Journal of Animal Science and Production. 82: 64-68.

Czech B, Wang Y, Szyda J. 2023. Genome-wide association study of heat stress response in Bos taurus. bioRxiv: 2023.06. 05.543663.

Dadousis C, Cipolat-Gotet C, Schiavon S, Bittante G, Cecchinato A. 2017a. Inferring individual cow effects, dairy system effects and feeding effects on latent variables underlying milk protein composition and cheese-making traits in dairy cattle. Journal of Dairy Research. 85: 87-97.

Dadousis C, Pegolo S, Rosa GJ, Bittante G, Cecchinato A. 2017b. Genome-wide association and pathway-based analysis using latent variables related to milk protein composition and cheesemaking traits in dairy cattle. Journal of Dairy Science. 100: 9085-9102.

Davenport KM, Bickhart DM, Worley K, Murali SC, Salavati M, Clark EL, Cockett NE, Heaton MP, Smith TP, Murdoch BM. 2022. An improved ovine reference genome assembly to facilitate in-depth functional annotation of the sheep genome. Gigascience 11: giab096.

De Pascale S, Caira S, Garro G, Mauriello R, Scaloni A, Cosenza G, Chianese L. 2022. Proteomic characterisation and phylogenetic derivation of ovine α_{s1}-CN B and α_{s1}-CN G genetic variants. International Dairy Journal. 131: 105387.

Dettori ML, Pazzola M, Petretto E, Vacca GM. 2020. Association analysis between SPP1, POFUT1 and PRLR gene variation and milk yield, composition and coagulation traits in Sarda sheep. Animals. 10: 1216.

Devani K, Crowley JJ, Plastow G, Orsel K, Valente TS. 2021. Genetic parameter estimations and genomic insights for teat and udder structure in young and mature Canadian Angus cows. Journal of Animal Science. 99: skab087.

Devani K, Plastow G, Orsel K, Valente TS. 2020. Genome-wide association study for mammary structure in Canadian Angus cows. Plos Oone. 15: e0237818.

Drögemüller C, Engensteiner M, Moser S, Rieder S, Leeb T. 2009. Genetic mapping of the belt pattern in Brown Swiss cattle to BTA3. Animal Genetics. 40: 225-229.

El-Halawany N, Abdel-Shafy H, Abd-El-Monsif AS, Abdel-Latif MA, Al-Tohamy AF, Abd El-Moneim OM. 2017. Genome-wide association study for milk production in Egyptian buffalo. Livestock Science. 198: 10-16.

Fang Z-H, Bovenhuis H, van Valenberg H, Martin P, Duchemin S, Huppertz T, Visker M. 2019. Genome-wide association study for α_{s1}-and α_{s2}-casein phosphorylation in Dutch Holstein Friesian. Journal of Dairy Science. 102: 1374-1385.

Fang Z-H, Visker M, Miranda G, Delacroix-Buchet A, Bovenhuis H, Martin P. 2016. The relationships among bovine αS-casein phosphorylation isoforms suggest different phosphorylation pathways. Journal of Dairy Science. 99: 8168-8177.

García-Gámez E, Gutierrez-Gil B, Sahana G, Sanchez JP, Bayón Y, Arranz JJ. 2012. GWA analysis for milk production traits in dairy sheep and genetic support for a QTN influencing milk protein percentage in the LALBA gene. Plos One. 7: e47782.

Gaspa G, Cesarani A, Correddu F, Congiu M, Dimauro C, Pauciullo A, Macciotta N 2022b. Heritability and genomic analysis of coagulation event in sheep milk. Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP) Technical and species orientated innovations in animal breeding, and contribution of genetics to solving societal challenges. Pp. 2928-2931.

Gaspa G, Correddu F, Cesarani A, Congiu M, Dimauro C, Pauciullo A, Macciotta NPP. 2022a. Multivariate and Genome-Wide Analysis of Mid-Infrared Spectra of Non-Coagulating Milk of Sarda Sheep Breed. Frontiers in Animal Science. 3: 889797.

Gaspa G, Serdino J, Manca M, Sorbolini S, Negrini R, Dimauro C, Macciotta N. 2016. Genome-wide association study of milk coagulation properties in dairy sheep. Journal of Animal Science. 94: 159-159.

Gilmour A, Gogel B, Cullis B, Welham S, Thompson R. 2015. ASReml user guide release 4.1 structural specification. Hemel hempsteadHempstead: VSN International Ltd.

Gutiérrez-Gil B, El-Zarei MF, Alvarez L, Bayón Y, De la Fuente LF, San Primitivo F, Arranz JJ. 2009. Quantitative trait loci underlying milk production traits in sheep. Animal Genetics. 40: 423-434.

Han R-X, Choi S-M, Kim M-Y, Quan YS, Kim B-C, Diao YF, Koqani R, Park C-S, Jin D-I. 2008. Identification of Bovine Pregnancy-Specific Whey Proteins using Two-Dimensional Gel Electrophoresis. Reproductive and Developmental Biology. 32: 255-261.

Hettinga KA 2019. Lactose in the dairy production chain. Lactose, Elsevier. Pp. 231-266.

Huang W, Peñagaricano F, Ahmad K, Lucey J, Weigel K, Khatib H. 2012. Association between milk protein gene variants and protein composition traits in dairy cattle. Journal of Dairy Science. 95: 440-449.

Jales Neto LH, Wicik Z, Torres GH, Takayama L, Caparbo VF, Lopes NH, Pereira AC, Pereira RM. 2020. Overexpression of SNTG2, TRAF3IP2, and ITGA6 transcripts

is associated with osteoporotic vertebral fracture in elderly women from community. Molecular Genetics & Genomic Medicine. 8: e1391.
Jayawardana J, Lopez-Villalobos N, McNaughton LR, Hickson RE. 2023. Genomic regions associated with milk composition and fertility traits in spring-calved dairy cows in New Zealand. Genes. 14: 860.
Jiang J, Ma L, Prakapenka D, VanRaden PM, Cole JB, Da Y. 2019. A large-scale genome-wide association study in US Holstein cattle. Frontiers in Genetics. 10: 442321.
Johnsson M. 2023. Genomics in animal breeding from the perspectives of matrices and molecules. Hereditas. 160: 20.
Kang X, Li M, Liu M, Liu S, Pan MG, Wiggans GR, Rosen BD, Liu GE. 2020. Copy number variation analysis reveals variants associated with milk production traits in dairy goats. Genomics. 112: 4934-4937.
Kelwick R, Desanlis I, Wheeler GN, Edwards DR. 2015. The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. Genome Biology 16: 1-16.
Kim S, Lim B, Cho J, Lee S, Dang C-G, Jeon J-H, Kim J-M, Lee J. 2021. Genome-wide identification of candidate genes for milk production traits in Korean Holstein cattle. Animals. 11: 1392.
Kozlowski M, Wilczak J, Motyl T, Gajewska M. 2011. Role of extracellular matrix and prolactin in functional differentiation of bovine BME-UV1 mammary epithelial cells. Polish Journal of Veterinary Sciences. 14:433-442.
Lander E, Kruglyak L. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genetics. 11: 241-247.
Lees N, Lees I. 2018. Competitive advantage through responsible innovation in the New Zealand sheep dairy industry. International Food and Agribusiness Management Review. 21: 505-524.
Li H, Wu XL, Tait Jr R, Bauck S, Thomas D, Murphy T, Rosa G. 2020. Genome-wide association study of milk production traits in a crossbred dairy sheep population using three statistical models. Animal Genetics. 51: 624-628.
Lopez-Villalobos N, King M, King J, R.S. M. 2017. Estimates of genetic parameters for direct and maternal genetic effects on weaning weights in Dairymeade sheep. Proceedings of the New Zealand Society of Animal Production. 77: 129-132.
Marina H, Pelayo R, Suárez-Vega A, Gutiérrez-Gil B, Esteban-Blanco C, Arranz J. 2021. Genome-wide association studies (GWAS) and post-GWAS analyses for technological traits in Assaf and Churra dairy breeds. Journal of Dairy Science. 104: 11850-11866.
Marina H, Reverter A, Gutiérrez-Gil B, Alexandre PA, Porto-Neto LR, Suárez-Vega A, Li Y, Esteban-Blanco C, Arranz J-J. 2020. Gene networks driving genetic variation in milk and cheese-making traits of Spanish Assaf sheep. Genes. 11: 715.
Marshall AC, Lopez-Villalobos N, Loveday SM, Ellis A, McNabb W. 2023. Modelling Lactation Curves for Dairy Sheep in a New Zealand Flock. Animals. 13: 349.
Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024a. Udder and teat morphology traits associated with milk production and somatic cell score in dairy sheep from a New Zealand flock. New Zealand Journal of Agricultural Research. 67: 348-360.
Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024b. Estimation of genetic parameters for production, composition and processability of milk from dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research: 1-18.

Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024c. Animal factors affecting the cheese-making properties and the heat coagulation time of milk from dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research: 1-18.

Marshall AC, Vigolo V, Di Marchi M, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2025. Effect of protein polymorphisms on milk composition, coagulation properties, and protein profile in dairy sheep. International Dairy Journal. 160: 106102. doi:https://doi.org/10.1016/j.idairyj.2024.106102

Martin FJ, Amode MR, Aneja A, Austine-Orimoloye O, Azov AG, Barnes I, Becker A, Bennett R, Berry A, Bhai J. 2023. Ensembl 2023. Nucleic Acids Research. 51: D933-D941.

McEwan J 2009. Current and future impact of DNA technologies on the New Zealand sheep industry. Proceedings of the New Zealand Society of Animal Production. Christchurch, New Zealand Society of Animal Production. Pp. 165-168.

McMahon DJ, Brown RJ. 1982. Evaluation of Formagraph for comparing rennet solutions. Journal of Dairy Science. 65: 1639-1642.

Mercier J-C. 1981. Phosphorylation of caseins, present evidence for an amino acid triplet code posttranslationally recognized by specific kinases. Biochimie. 63: 1-17.

Mi B-n, Zhang L-g, Bai U, Guo Y-l, Wang C-w, Xu Q-z, Feng S, Li G-p, Su X-h, Zhang L. 2021. Genome-wide association study of milk production traits in Dairy Meade sheep. Acta Veterinaria et Zootechnica Sinica. 52: 3294-3303.

Moatsou G, Samolada M, Katsabeki A, Anifantakis E. 2004. Casein fraction of ovine milk from indigenous Greek breeds. Le Lait. 84: 285-296.

Mohapatra A, Shinde AK, Singh R. 2019. Sheep milk: A pertinent functional food. Small Ruminant Research. 181: 6-11.

Moioli B, Steri R, Napolitano F, Catillo G. 2015. A genome wide scan highlights differences in the genetic architecture of fat and protein contents in dairy sheep. Small Ruminant Research. 131: 21-28.

Moon JY, Choi SJ, Heo CH, Kim HM, Kim HS. 2017. α-Syntrophin stabilizes catalase to reduce endogenous reactive oxygen species levels during myoblast differentiation. The FEBS Journal. 284: 2052-2065.

Ni J, Xian M, Ren Y, Yang L, Li Y, Guo S, Ran B, Hu J. 2024. Whole-genome resequencing reveals candidate genes associated with milk production trait in Guanzhong dairy goats. Animal Genetics. 55: 168-172.

Noce A, Pazzola M, Dettori ML, Amills M, Castelló A, Cecchinato A, Bittante G, Vacca GM. 2016. Variations at regulatory regions of the milk protein genes are associated with milk traits and coagulation properties in the Sarda sheep. Animal Genetics. 47: 717-726.

Pazzola M, Vacca GM, Paschino P, Bittante G, Dettori ML. 2021. Novel Genes Associated with Dairy Traits in Sarda Sheep. Animals. 11: 2207.

Pazzola M. 2019. Coagulation Traits of Sheep and Goat Milk. Animals. 9:540.

Pedrosa VB, Schenkel FS, Chen S-Y, Oliveira HR, Casey TM, Melka MG, Brito LF. 2021. Genomewide association analyses of lactation persistency and milk production traits in Holstein cattle based on imputed whole-genome sequence data. Genes. 12: 1830.

Pirisi A, Piredda G, Papoff CM, Di Salvo R, Pintus S, Garro G, Ferranti P, Chianese L. 1999. Effects of sheep α_{s1}-casein CC, CD and DD genotypes on milk composition and cheesemaking properties. Journal of Dairy Research. 66: 409-419.

Riley L, Gardiner-Garden M, Thomson P, Wynn P, Williamson P, Raadsma H, Sheehy P. 2010. The influence of extracellular matrix and prolactin on global gene expression

profiles of primary bovine mammary epithelial cells in vitro. Animal Genetics. 41: 55-63.

Sahana G, Guldbrandtsen B, Thomsen B, Holm L-E, Panitz F, Brøndum RF, Bendixen C, Lund MS. 2014. Genome-wide association study using high-density single nucleotide polymorphism arrays and whole-genome sequences for clinical mastitis traits in dairy cattle. Journal of Dairy Science. 97: 7258-7275.

Sanchez M-P, Rocha D, Charles M, Boussaha M, Hozé C, Brochard M, Delacroix-Buchet A, Grosperrin P, Boichard D. 2021. Sequence-based GWAS and post-GWAS analyses reveal a key role of SLC37A1, ANKH, and regulatory regions on bovine milk mineral content. Scientific Reports. 11: 7537.

Scholtens M, Jiang A, Smith A, Littlejohn M, Lehnert K, Snell R, Lopez-Villalobos N, Garrick D, Blair H. 2020. Genome-wide association studies of lactation yields of milk, fat, protein and somatic cell score in New Zealand dairy goats. Journal of Animal Science and Biotechnology. 11: 1-14.

Schopen G, Visker M, Koks P, Mullaart E, Van Arendonk J, Bovenhuis H. 2011. Whole-genome association study for milk protein composition in dairy cattle. Journal of Dairy Science. 94: 3148-3158.

Selvaggi M, Laudadio V, Dario C, Tufarelli V. 2014. Investigating the genetic polymorphism of sheep milk proteins: a useful tool for dairy production. Journal of the Science of Food and Agriculture. 94: 3090-3099.

Sheng H, Zhang J, Pan C, Wang S, Gu S, Li F, Ma Y, Ma Y. 2023. Genome-wide identification of bovine ADAMTS gene family and analysis of its expression profile in the inflammatory process of mammary epithelial cells. International Journal of Biological Macromolecules. 244: 125304.

Shin D-H, Lee H-J, Cho S, Kim HJ, Hwang JY, Lee C-K, Jeong J, Yoon D, Kim H. 2014. Deleted copy number variation of Hanwoo and Holstein using next generation sequencing at the population level. BMC Genomics. 15: 1-16.

Sodeland M, Grove H, Kent M, Taylor S, Svendsen M, Hayes BJ, Lien S. 2011. Molecular characterization of a long range haplotype affecting protein yield and mastitis susceptibility in Norwegian Red cattle. BMC Genetics. 12: 1-10.

Sutera A, Riggio V, Mastrangelo S, Di Gerlando R, Sardina M, Pong-Wong R, Tolone M, Portolano B. 2019. Genome-wide association studies for milk production traits in Valle del Belice sheep using repeated measures. Animal Genetics. 50: 311-314.

Sutera AM, Moscarelli A, Mastrangelo S, Sardina MT, Di Gerlando R, Portolano B, Tolone M. 2021. Genome-wide association study identifies new candidate markers for somatic cells score in a local dairy sheep. Frontiers in Genetics. 12: 409.

Thornell IM, Bevensee MO. 2015. Regulators of Slc4 bicarbonate transporter activity. Frontiers in Physiology. 6: 166.

Turner SD. 2014. qqman: an R package for visualizing GWAS results using QQ and manhattan plots. Biorxiv: 005165.

Vigolo V, Franzoi M, Cendron F, Salvadore G, Penasa M, Cassandro M, De Marchi M. 2022. Characterization of the genetic polymorphism linked to the β-casein A1/A2 alleles using different molecular and biochemical methods. Journal of Dairy Science. 105: 8946-8955.

Wang B, Hou L, Zhou W, Liu H, Tao W, Wu W, Niu P, Zhang Z, Zhou J, Li Q. 2021. Genome-wide association study reveals a quantitative trait locus and two candidate genes on Sus scrofa chromosome 5 affecting intramuscular fat content in Suhuai pigs. Animal. 15: 100341.

Wedholm A, Larsen LB, Lindmark-Månsson H, Karlsson A, Andrén A. 2006. Effect of protein composition on the cheese-making properties of milk from individual dairy cows. Journal of Dairy Science. 89: 3296-3305.

Wu X, Lund MS, Sahana G, Guldbrandtsen B, Sun D, Zhang Q, Su G. 2015. Association analysis for udder health based on SNP-panel and sequence data in Danish Holsteins. Genetics Selection Evolution. 47: 1-14.

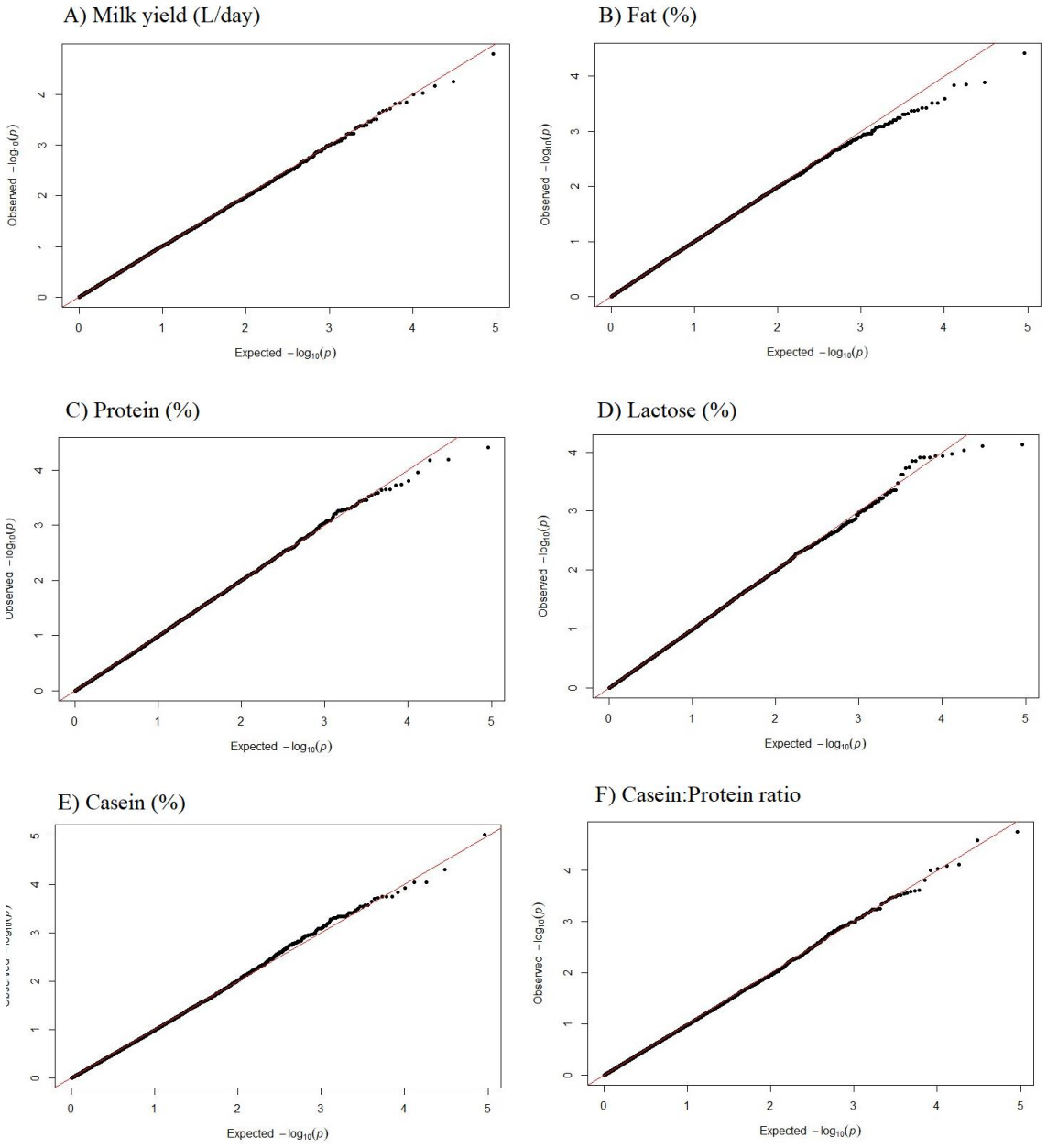
Yang J, Lee SH, Goddard ME, Visscher PM. 2011. GCTA: a tool for genome-wide complex trait analysis. The American Journal of Human Genetics. 88: 76-82.

Yang X, Ren S, Rehman ZU, Wang X, Yin X, Sun Y, Wan X, Chen H. 2022. Molecular characterization, expression, and functional identification of TANK-binding kinase 1 (TBK1) of the cow (Bos taurus) and goat (Capra hircus). Developmental & Comparative Immunology. 133: 104444.

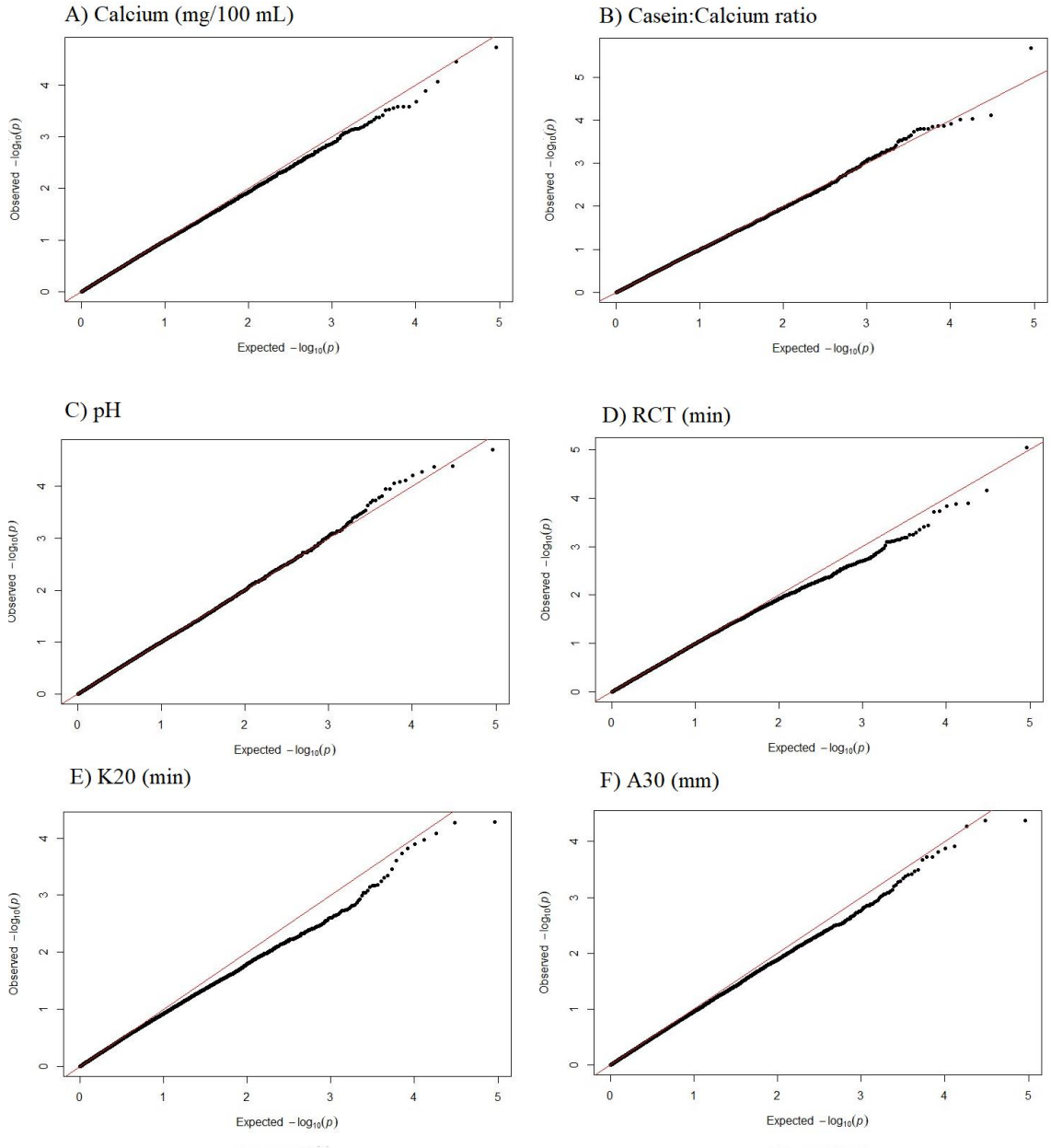
Zhang Q, Guldbrandtsen B, Thomasen JR, Lund MS, Sahana G. 2016. Genome-wide association study for longevity with whole-genome sequencing in 3 cattle breeds. Journal of Dairy Science. 99: 7289-7298.

Zhou C, Li C, Cai W, Liu S, Yin H, Shi S, Zhang Q, Zhang S. 2019. Genome-wide association study for milk protein composition traits in a Chinese Holstein population using a single-step approach. Frontiers in Genetics. 10: 72.

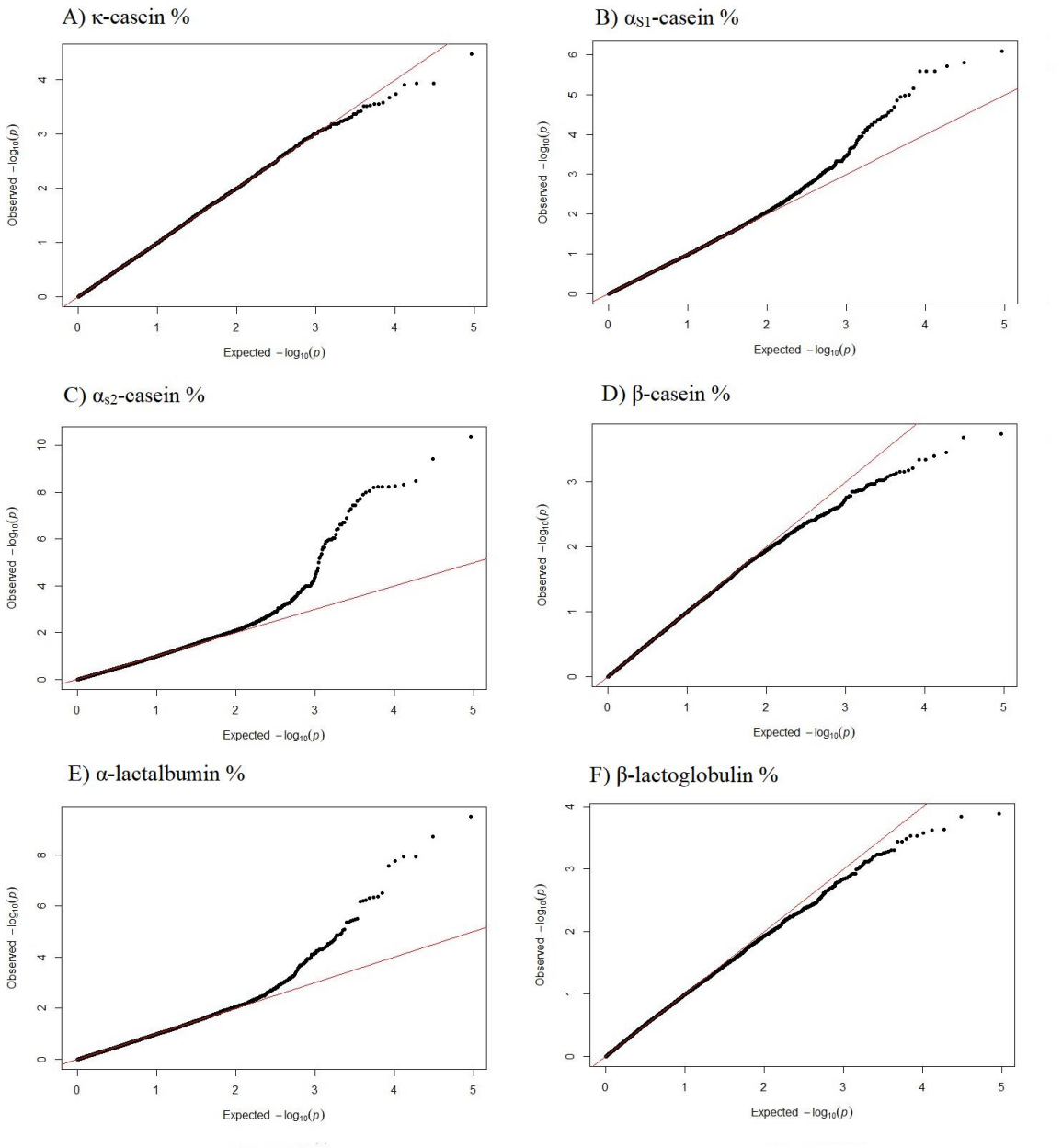
Appendix 3 (Supplementary information for Chapter 7)



Appendix 3 Figure 1. Quantile-quantile (QQ) plots of the data shown in the Manhattan plots of milk yield, fat, protein, lactose, and casein contents, and ratio of casein to protein, in A, B, C, D, E, and F, respectively.



Appendix 3 Figure 2. Quantile-quantile (QQ) plots of the data shown in the Manhattan plots of calcium content, ratio of casein to calcium, pH, and milk coagulation properties (RCT, K20, and A30), in A, B, C, D, E, and F, respectively.



Appendix 3 Figure 3. Quantile-quantile (QQ) plots of the data shown in the Manhattan plots of content (% in total protein of sheep milk) of κ-, α_{s1}-, α_{s2}-, β- casein, α-lactalbumin, and β-lactoglobulin, in A, B, C, D, E, and F, respectively.

Appendix 3 Table 1. List of candidate genes associated with the respective studied traits, gene annotations, and gene ontology terms of biological processes or molecular functions.

Gene	Annotation	GO Term (biological process, otherwise molecular function)	Trait(s) associated
ADAMTS20	ADAM metallopeptidase with thrombospondin type 1 motif 20.	GO:0006508, proteolysis. GO:0030198, extracellular matrix organization.	α-LA
ADAMTS3	ADAM metallopeptidase with thrombospondin type 1 motif 3.	GO:0001701, in utero embryonic development. GO:0006508, proteolysis. GO:0016485, protein processing.	α_{s2}-CN
ADCY6	Adenylate cyclase type 6; Catalyzes the formation of the signaling molecule cAMP in response to G-protein signaling.	GO:0006171, cAMP biosynthetic process.	α-LA
ANTXR2	Anthrax toxin receptor; Belongs to the ATR family.	Molecular GO:0038023, signaling receptor activity. Molecular GO:0046872, metal ion binding.	α_{s2}-CN
ARHGAP24	Rho GTPase activating protein 24.	GO:0007165, signal transduction.	α_{s2}-CN
AVPR1A	Vasopressin V1a receptor; Receptor for arginine vasopressin. The activity of this receptor is mediated by G proteins which activate a phosphatidyl- inositol-calcium second messenger system; Belongs to the G-protein coupled receptor 1 family. Vasopressin/oxytocin receptor subfamily.	GO:0007186, G protein-coupled receptor signaling pathway. Molecular GO:0005000, vasopressin activated calcium mobilizing receptor activity.	α-LA
BMP2K	Protein kinase domain-containing protein.	GO:0006468, protein phosphorylation. GO:0030500, regulation of bone mineralization. Molecular GO:0004672, protein kinase activity.	α_{s1}-CN and α_{s2}-CN

		Molecular GO:0005524, ATP binding. Molecular GO:0019208, phosphatase regulator activity.	
CACNB3	Calcium voltage-gated channel auxiliary subunit beta 3.	GO:0006816, calcium ion transport. GO:0070588, calcium ion transmembrane transport. GO:0006811, monoatomic ion transport.	α-LA
CCDC158	Coiled-coil domain containing 158.	-	α_{s1}-CN and α_{s2}-CN
CCDC65	Coiled-coil domain containing 65.	GO:0003352, regulation of cilium movement.	α-LA
CCNG2	Cyclin G2; Belongs to the cyclin family.	GO:0051726, regulation of cell cycle.	α_{s2}-CN
CCNI	Cyclin I; Belongs to the cyclin family.	-	α_{s2}-CN
CCNT1	Cyclin T1; Belongs to the cyclin family.	GO:0019901, protein kinase binding. GO:0016538, cyclin-dependent protein serine/threonine kinase regulator activity.	α-LA
CDS1	Phosphatidate cytidylyltransferase; Provides CDP-diacylglycerol, an important precursor for the synthesis of phosphatidylinositol, phosphatidylglycerol, and cardiolipin.	Molecular GO:0016772, transferase activity, transferring phosphorus-containing groups. GO:0016024, CDP-diacylglycerol biosynthetic process.	α_{s2}-CN
CFAP299	Uncharacterized protein.	-	α_{s1}-CN and α_{s2}-CN
CNTN1	Contactin 1.	GO:0010765, positive regulation of sodium ion transport. GO:0010467, gene expression. GO:0032289, central nervous system myelin formation. Molecular GO:0005515, protein binding. Molecular GO:0030246, carbohydrate binding.	MY and α-LA
CPNE8	Copine 8.	Molecular GO:0005544, calcium-dependent phospholipid binding.	α-LA

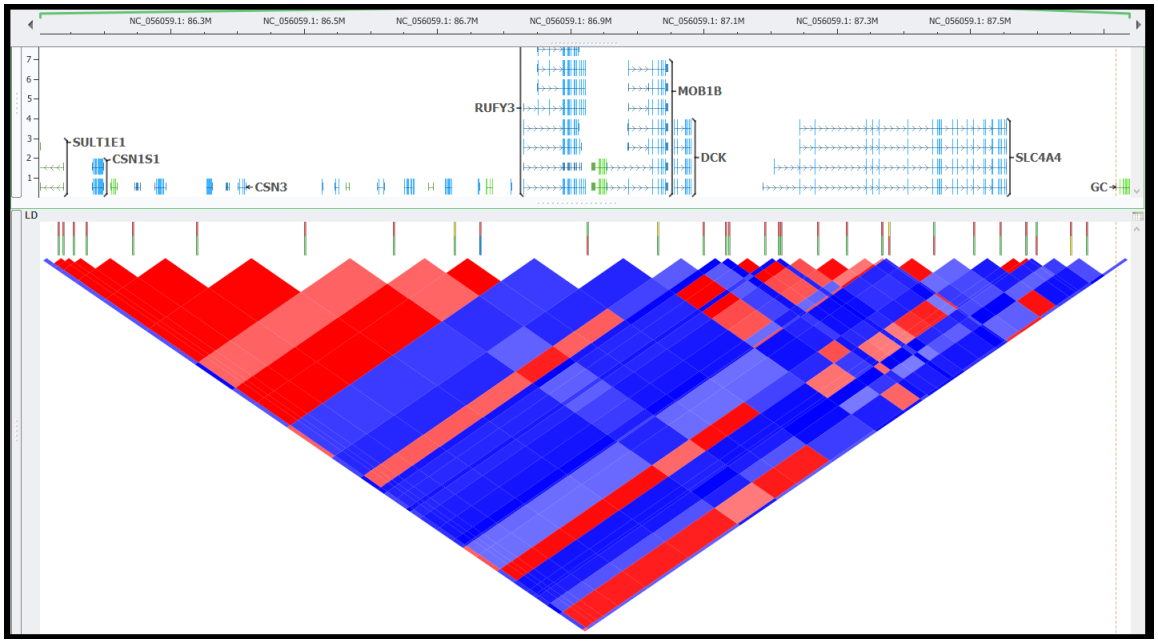
CTSL	Cathepsin L1; Belongs to the peptidase C1 family.	GO:0002250, adaptive immune response. GO:0006508, proteolysis. GO:0051603, proteolysis involved in protein catabolic process. GO:0097067, cellular response to thyroid hormone stimulus.	CSN:PP
CXCL10	C-X-C motif chemokine.	GO:0006955, immune response. GO:0006952, defense response.	α_{s2}-CN
CXCL13	SCY domain-containing protein.	GO:0006955, immune response. GO:0006952, defense response.	α_{s2}-CN
DCK	Deoxycytidine kinase.	GO:1901293, nucleoside phosphate biosynthetic process. Molecular GO:0042803, protein homodimerization activity.	MY and α_{s2}-CN
EMB	Embigin.	GO:0007155, cell adhesion GO:0007411, axon guidance Molecular GO:0005515, protein binding	pH
EPHA5	Receptor protein-tyrosine kinase.	GO:0006468, protein phosphorylation GO:0007169, transmembrane receptor protein tyrosine kinase signaling pathway Molecular GO:0004672, protein kinase activity Molecular GO:0005515, protein binding	α_{s2}-CN
FBP1	Fructose-1,6-bisphosphatase 1; Catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate in the presence of divalent cations, acting as a rate-limiting enzyme in gluconeogenesis. Plays a role in	GO:0005975, carbohydrate metabolic process GO:0006094, gluconeogenesis GO:0016311, dephosphorylation	CSN:PP

	regulating glucose sensing and insulin secretion of pancreatic beta-cells. Appears to modulate glycerol gluconeogenesis in liver.		
FBP2	Fructose-bisphosphatase 2; Belongs to the FBPase class 1 family.	GO:0005975, carbohydrate metabolic process GO:0006094, gluconeogenesis	CSN:PP
FKBP	FKBP prolyl isomerase like.	GO:0016853, isomerase activity	α-LA
FRAS1	Fraser extracellular matrix complex subunit 1.	GO:0015031, protein transport GO:0002009, morphogenesis of an epithelium	α_{s2}-CN
GC	GC vitamin D binding protein.	GO:0042359, vitamin D metabolic process GO:0051180, vitamin transport	α_{s2}-CN
GK2	Glycerol kinase 2; Belongs to the FGGY kinase family.	GO:0005975, carbohydrate metabolic process GO:0006072, glycerol-3-phosphate metabolic process GO:0016310, phosphorylation GO:0007283, spermatogenesis	α_{s1}-CN and α_{s2}-CN
HNRNPDL	Uncharacterized protein.	Molecular GO:0003676, nucleic acid binding	α_{s2}-CN
LRRK2	Leucine rich repeat kinase 2.	GO:0000165, MAPK cascade GO:0001933, negative regulation of protein phosphorylation GO:0001934, positive regulation of protein phosphorylation GO:0006468, protein phosphorylation Molecular GO:0004672, protein kinase activity Molecular GO:0004674, protein serine/threonine kinase activity GO:0005515, protein binding	α-LA
MAPK10	Mitogen-activated protein kinase.	GO:0006468, protein phosphorylation	α_{s2}-CN

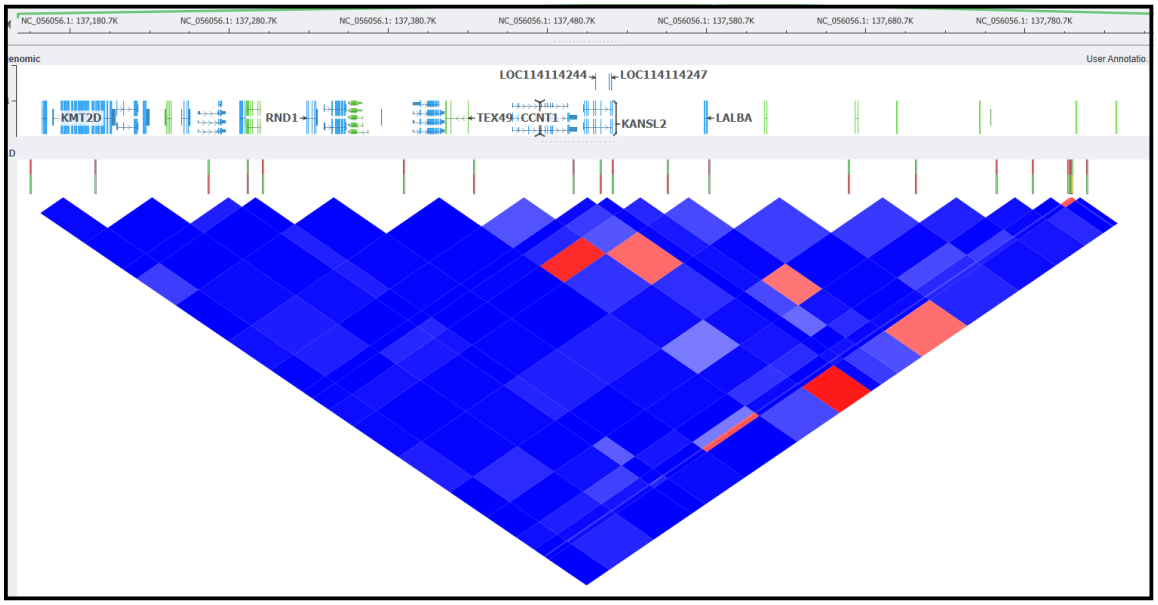
MRPL1	Uncharacterized protein.	GO:0006412, translation	α_{s2}-CN
MUC19	Mucin 19, oligomeric	GO:0005515, protein binding	α-LA
NAA11	N(alpha)-acetyltransferase 11, NatA catalytic subunit.	GO:0018002, N-terminal peptidyl-glutamic acid acetylation GO:0017198, N-terminal peptidyl-serine acetylation	α_{s1}-CN and α_{s2}-CN
NKX6-1	Homeobox domain-containing protein.	GO:0030154, cell differentiation GO:0006357, regulation of transcription by RNA polymerase II	α_{s2}-CN
NPFFR2	G_protein_recep_F1_2 domain-containing protein.	GO:0007186, G protein-coupled receptor signaling pathway GO:0043408, regulation of MAPK cascade GO:0045761, regulation of adenylate cyclase activity GO:2000479, regulation of cAMP-dependent protein kinase activity (PKA)	α_{s2}-CN
NUP54	Nucleoporin 54.	GO:0005635, nuclear envelope	α_{s2}-CN
PDZRN4	PDZ domain containing ring finger 4.	GO:0005515, protein binding	MY and α-LA
RASGEF1B	RasGEF domain family member 1B.	GO:0007264, small GTPase mediated signal transduction	α_{s2}-CN
RND1	Rho family GTPase 1.	GO:0007264, small GTPase mediated signal transduction GO:0062176, R-loop processing	α-LA
SCD5	FA_desaturase domain-containing protein; Belongs to the fatty acid desaturase type 1 family.	GO:0006629, lipid metabolic process	α_{s2}-CN
SHROOM3	ASD2 domain-containing protein.	GO:0005515, protein binding	α_{s2}-CN
SLC2A13		GO:0055085, transmembrane transport	α-LA

	Solute carrier family 2 member 13; Belongs to the major facilitator superfamily. Sugar transporter (TC 2.A.1.1) family.	GO:0015798, myo-inositol transport GO:1902004, positive regulation of amyloid-beta formation	
SLC38A9	Solute carrier family 38 member 9.	GO:0003333, amino acid transmembrane transport GO:0032008, positive regulation of TOR signaling	RCT
SLC4A4	Anion exchange protein.	GO:0006814, sodium ion transport GO:0006820, monoatomic anion transport GO:0015701, bicarbonate transport GO:0035725, sodium ion transmembrane transport GO:0045821, positive regulation of glycolytic process GO:0051453, regulation of intracellular pH	α_{s1}-CN and α_{s2}-CN
SNTG2	Syntrophin gamma 2.	GO:0005515, protein binding GO:0005198, structural molecule activity	Calcium
SMPIP11	Sperm microtubule inner protein 11	-	α-LA
SRGAP1	SLIT-ROBO Rho GTPase activating protein 1.	GO:0007165, signal transduction Molecular GO:0005515, protein binding	α-LA
TBC1D30	TBC1 domain family member 30.	GO:1902018, negative regulation of cilium assembly	α-LA
TBK1	TANK binding kinase 1.	GO:0006468, protein phosphorylation GO:0004674, protein serine/threonine kinase activity GO:0002218, activation of innate immune response GO:0010468, regulation of gene expression Molecular GO:0051219, phosphoprotein binding Molecular GO:0042802, identical protein binding	Casein and α-LA

TMEM150C	Transmembrane protein 150C.	GO:0071260, cellular response to mechanical stimulus	α_{s2}-CN
TMPRSS11D	Transmembrane serine protease 11D; Belongs to the peptidase S1 family.	GO:0006508, proteolysis Molecular GO:0004252 serine-type endopeptidase activity	α_{s2}-CN
USO1	USO1 vesicle transport factor.	GO:0006886, intracellular protein transport GO:0016192, vesicle-mediated transport GO:1900076, regulation of cellular response to insulin stimulus Molecular GO:0005515, protein binding	α_{s1}-CN and α_{s2}-CN
WAPL	WAPL cohesin release factor.	GO:0008156, negative regulation of DNA replication	α_{s1}-CN



Appendix 3 Figure 4. Linkage disequilibrium region between the casein cluster (CSN1S1, CSN1S2, CSN2, CSN3), DCK, SLC4A4, and GC genes in SVS software after data quality control processes.



Appendix 3 Figure 5. Linkage disequilibrium region between CCNT1 and LALBA genes in SVS software after data quality control processes.

Appendix 3 Table 2. Estimates of variance components[1] and heritabilities (h^2) of milk production, composition, protein composition, milk coagulation properties, and milk pH, with their respective standard errors (SE) obtained from genomic-based (SNP) analysis.

Trait	σ_a^2	SE	σ_e^2	SE	σ_p^2	SE	h^2	SE
Milk yield (mL/day)	11737	3937	5512	2536	17250	2378	0.68	0.16
Fat (%)	8.44	3.15	4.91	2.11	13.36	1.84	0.63	0.18
Protein (%)	7.85	3.21	4.43	2.14	12.29	1.75	0.64	0.20
Lactose (%)	0.34	0.27	1.00	0.25	1.34	0.17	0.26	0.19
Casein (%)	4.15	1.77	2.61	1.21	6.76	0.96	0.61	0.20
Casein:Protein	0.67	0.25	0.35	0.17	1.01	0.14	0.66	0.19
Calcium (mg/100 ml)	68.39	28.95	56.86	20.74	125.26	16.88	0.55	0.19
Casein:Calcium	44.02	24.81	65.74	19.81	109.76	14.27	0.40	0.20
κ-casein (% in total protein)	0.06	0.03	0.07	0.02	0.13	0.02	0.46	0.19
α_{s1}-casein (% in total protein)	0.84	0.36	0.57	0.25	1.41	0.20	0.60	0.20
α_{s2}-casein (% in total protein)	0.79	0.31	0.51	0.21	1.30	0.18	0.61	0.19
β-casein (% in total protein)	0.00	0.06	0.53	0.09	0.53	0.06	-	-
α-lactalbumin (% in total protein)	0.08	0.03	0.03	0.02	0.11	0.02	0.70	0.17
β-lactoglobulin (% in total protein)	0.14	0.06	0.13	0.04	0.27	0.03	0.52	0.18
RCT (min)	1.04	0.91	3.63	0.85	4.67	0.57	0.22	0.19
K20 (min)	0.00	0.12	0.75	0.13	0.75	0.09	-	-
A30 (mm)	0.00	4.08	28.73	5.07	28.73	3.39	-	-
pH	0.00	0.00	0.00	0.00	0.00	0.00	0.90	0.13

[1] σ_a^2= additive genetic variance; σ_e^2= residual variance; σ_p^2= total phenotypic variance.

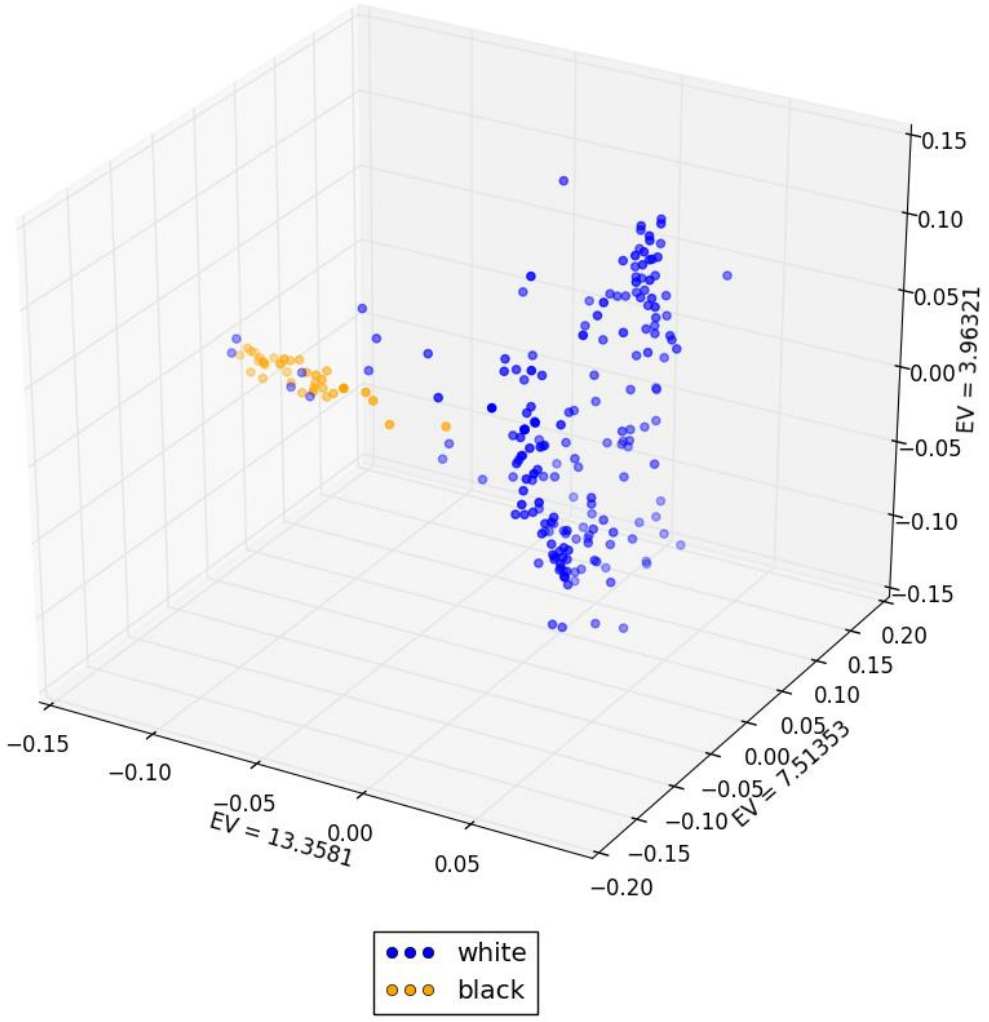
Appendix 3 Table 3. Estimates of genomic correlations (r_g) and respective standard errors (SE) between rennet coagulation time (RCT) and milk production, composition, protein composition, and milk pH.

Trait[1]	MY	FP	PP	LP	CSNP	CSNP:PP	Calcium	CSNP:Calcium	κ-CN	α_{s1}- CN	α_{s2}- CN	β-CN	α-LA	β-LG	pH
r_g	-0.34	0.01	0.76	-0.86	0.65	-0.77	-	0.92	-	0.73	-	-	-0.50	-0.57	0.88
SE	0.22	0.24	0.18	0.22	0.23	0.15	-	0.25	-	0.30	-	-	0.23	0.23	0.13

[1] MY= daily milk yield; FP= fat percentage; PP= protein percentage; LP= lactose percentage; CSNP= casein percentage; CSNP:PP= ratio of casein percentage to protein percentage; CSNP:Calcium= ratio of casein percentage to calcium content; κ-CN= κ-casein (% in total protein), α_{s1}- CN= α_{s1}-casein (% in total protein); α_{s2}- CN= α_{s2}-casein (% in total protein); β-CN= β-casein (% in total protein); α-LA= α-lactalbumin (% in total protein); β-LG= β-lactoglobulin (% in total protein).

Appendix 3 Table 4. Number of animals included in the ASReml pedigree file for ewe phenotype adjustment before GWAS analyses in GCTA software.

Pedigree file for ASReml run							
Animals	237						
Sires	19	Sires of Sire	0	Sires of Dam	10	Generations (Sire side)	1-2
Dams	100	Dams of Sire	0	Dams of Dam	23	Generations (Dam side)	1-4
Ewes	147						



Appendix 3 Figure 6. A three-dimensional scatter plot for principal components identifying coat colour as the first principal component (PC1, EV= 13.35) in a New Zealand dairy sheep flock. The blue dots represent the white sheep, and the yellow dots represent the black sheep.

Chapter 8

General discussion

8.1 Introduction

The dairy sheep industry is relatively new in New Zealand, and genetic and genomic studies on milk production, composition, and processability traits were lacking. Such studies are crucial for supporting and advancing the sector, as genetic improvement with implementing a selection index could be one way to increase production efficiently and sustainably.

The main aim of this thesis was to investigate the genetic parameters and genomic architecture of milk traits in a flock of dairy sheep. The test-day records on milk production, composition, and processability were taken during the 2021-2022 milk production season of a small flock of 169 ewes farmed in an extensive grazing system (see Tables 8.1 and 8.2 for clarification on the data structure). The dairy ewes were milked for artisan cheese production, after the start of lamb weaning, in October 2021. Milking frequency was twice-a-day after weaning and this frequency was dropped to once-a-day in November, until flock dry-off in mid-February, at the farmer's discretion. The participating farm is a vertically integrated business, controlling all stages of production until the final products (artisan cheeses) reach retail. The farm has been pedigree-recording over decades, with the selection of replacement ewes and rams done by the farmer, selection has been primarily based on ewe temperament, health, and dairy conformation. The breed was developed from crossing between East-Friesian sheep and meat breeds from New Zealand. Historically, the farm has provided lambs (both ewe and ram lambs) to other farms on the North Island, which may have created some genetic link between this flock and other farms in New Zealand.

Table 8.1. Distribution of ewes by coat-colour, age, and litter size, in the studied flock during the 2021-2022 production season (dataset used in Chapters 3, 4, and 6).

Coat-colour:	White				Black				
Age:	1 y.o.	2 y.o.	3 y.o.	≥ 4 y.o.	1 y.o.	2 y.o.	3 y.o.	≥ 4 y.o.	Total:
1 lamb	16	18	7	9	3	2	3	8	66
≥ 2 lambs	7	26	21	32	0	1	8	8	103
Total:	23	44	28	41	3	3	11	16	169

Table 8.2. Distribution of ewes by coat-colour, age, and litter size, in the studied flock during the 2021-2022 production season (dataset used in Chapters 5 and 7).

Coat-colour:	White				Black				
Age:	1 y.o.	2 y.o.	3 y.o.	≥ 4 y.o.	1 y.o.	2 y.o.	3 y.o.	≥ 4 y.o.	Total:
1 lamb	13	15	7	9	1	2	3	5	55
≥ 2 lambs	6	25	21	27	0	1	6	6	92
Total:	19	40	28	36	1	3	9	11	147

8.2 Major findings from this thesis and applications

8.2.1 Milk production

The first part of this thesis reported the average flock production before conducting the genetic and genomic analyses, to explore variation in the dataset and the ewe factors affecting the lactation curves. The studied flock showed, overall, relatively low total milk yield per ewe and a sharp decline in the lactation curves. This was suggested to be largely a consequence of the extensive nature of this pasture-based farming system, where the weather/season strongly influences the lactation curves. The low production recorded was also suggested to be a consequence of the long exclusive lamb suckling period that lasted a minimum of 4 weeks at this farm, and the peak milk production of early lactation might have been missed.

These environmental and management factors might have masked the true genetic potential of some dairy sheep of this flock for milk production. To address this issue, feed management in pasture systems should be optimized, and, ideally, genotype x environment interaction should be investigated across different farms, to truly isolate genetic factors influencing milk production. However, the overall low milk production could also indicate a need for dairy sheep genetics that could be better suited to an extensive grazing system in New Zealand's climatic conditions.

Another challenge noted throughout this research were the difficulties related to the measurement of milk volume and milk composition in dairy sheep. This was found to be highly laborious when daily milk volumes per ewe are small (specially in mid to late lactation stages). Also, appropriate sampling equipment (ICAR certified milk meters for sheep) is expensive, which might not be justified for small dairy sheep businesses. Therefore, it is sensible that small business farms, such as the one described here, have

focused on selective breeding of dairy sheep on animal health and dairy conformation, to reduce production costs. Methods for recording sheep milk traits would need to become more affordable to be implemented in small farms for selective breeding on milk yield traits. Whereas larger dairy sheep companies and breeds of economic importance for export revenue should consider incorporating milk production and milk quality traits, as well as other traits of economical and sustainable importance in a selection index.

It is appealing for the dairy sheep sector to adopt a structure similar to the New Zealand dairy cattle industry, with a unified national breeding objective and a standardized selection index across herds, focusing on selection pressure on sires that are progeny tested and with genetic material disseminated through artificial insemination. However, this approach is challenged by the high costs of milk recording relative to the value of ewes, especially on a large commercial scale. Additionally, there are challenges with artificial insemination in sheep, and tracking pedigree information in sheep is more complex due to natural mating in paddocks, fence-jumping, and the birth of multiple lambs, necessitating the use of DNA parentage testing. To justify these costs, the value of dairy sheep products (revenue) needs to be high, either through the marketing of premium dairy products (Lees and Lees 2018)) or through the combined high value of lambs. The selection index for sheep should also consider traits beyond milk attributes, including udder conformation, disease resistance (some of which are already included in selection indexes for meat and dual-purpose sheep), and traits related to the environment and to animal welfare (Johnson et al. 2022).

This thesis also confirmed that factors related to the ewes, such as parity number and litter size, and management decisions (such as milking frequency) influenced the lactation curves which could be reflected in the processability of milk. Coat-colour variety was not a significant factor, but there was a limited number of black ewes. These factors (as well as breed and genetic variety, where applicable) should always be accounted for in genetic animal evaluation schemes, and even in future research in the field of food science and technology, which would help to properly identify intrinsic raw milk characteristics that would be desirable for processing.

8.2.2 Milk processability

The ewe factors, which included parity number (ewe age at lambing), coat colour, litter size, stage of lactation, and deviation from the median lambing date of the flock significantly affected milk composition and processability traits in the studied flock.

The stage of lactation, which is naturally confounded with the time of the year (seasonality) in pasture-based systems, was found to be the strongest factor influencing the properties of milk for cheese-making and its heat stability. This suggests that sheep milk processing plants should be adjusting their methods accordingly if compositional changes in the milk are detected in the vat. Key mechanisms that may contribute to the observed variation in milk processability include changes in milk pH, which is known to be largely determined by minerals in the milk's soluble phase, and milk buffering capacity. The ratios of casein to calcium and to total protein could be particularly important, while a higher somatic cell count could be linked to impaired milk processability.

Also, there could be an opportunity for genetic selection of dairy sheep for more stable milk production and composition throughout the lactation/milking season, to avoid drastic changes in milk composition and sharp decreases in milk quality in late lactation. It is worth investigating the inclusion of lactation persistency in animal selection. But again, in grazing systems, weather conditions, and feed management strongly influence the lactation curves of dairy animals, especially for those farmed on pasture (Sevi et al. 2004). Other management factors, such as milking practices (such as frequency, over-milking, hygiene) and dry-off date, could also be strongly influencing the udder and mammary gland health and therefore the characteristics of late-lactation milk.

Despite having observed milk compositional and processability changes throughout the season/lactation, the rennet coagulation ability of the milk samples from individual ewes were, in general, satisfactory, which was evidenced through the relatively fast coagulation and strong curd firmness measured, confirming the good attributes of sheep milk for cheese production. Also, a relatively high proportion of milk from individual sheep milk samples was converted into cheese in the laboratory cheese-making method. The heat-induced coagulation time of the individual sheep milk samples was also investigated, but the literature on sheep milk heat coagulation time was limited on individual ewe milk samples, although it is known that sheep milk has overall lower heat-stability compared to cow milk due to its intrinsic physicochemical properties (Raynal-Ljutovac et al. 2007)

making it more challenging to transform sheep milk into products that require high-temperature treatment such as milk powder and UHT beverages, especially in late-lactation.

In this study, it was noted that the milk processability traits (both for cheese-making and for heat-stability) are even more laborious to obtain than composition traits and are likely not feasible to be recorded routinely with hundreds of milk samples obtained per day. The food scientists, food technologists, and engineers could, in a multidisciplinary effort, develop faster and accurate ways of assessing milk processability traits for large recording schemes that require several milk samples of small volume. Alternatively, model equations could be developed to predict processability traits on milk composition, if they can be proven to be of high accuracy, which is challenging to achieve due to the complex nature of milk physical-chemistry, interactions, and balances between its components. Additionally, in the field of animal breeding, a training population could be used for genomic predictions on these laborious milk processability traits.

8.2.3 Milk protein profile

The quantity of each protein (κ-CN, α_{s1}-CN, α_{s2}-CN, β-CN, α-LA, and β-LG) in total protein were analysed in sheep milk samples from mid-late lactation of once-a-day milking, using RP-HPLC. The analyses also allowed the observation of polymorphisms for each protein. The protein analyses of the individual sheep milk samples from the studied flock provided additional insights on other effects that could be associated with composition and processability of sheep milk, at the phenotypic level. A minimum of two polymorphisms per protein were observed in the RP-HPLC in this small dairy sheep population.

The main finding was that polymorphism of β-lactoglobulin was significantly associated with sheep milk heat stability (phenotypically), the AB type of β-lactoglobulin was more heat stable than AA. The protein genotypes were not confirmed with DNA PCR; however, the variants of β-LG likely were either A or B, because C is very uncommon and the elution times of these polymorphisms were similar to a previous study that reported A and B (Trujillo et al. 2000). Also, some proteins were significantly ($p < 0.05$) associated with milk composition and protein profile. However, the milk processability traits were more linked to differences in the content of protein fractions rather than to the direct effect of

protein genotypes. Another finding was that the polymorphisms of casein proteins (α_{s1}-CN, α_{s2}-CN, and β-CN) seemed to be inherited together as a haplotype, which is likely due to linkage disequilibrium and close location of the genes (CSN1S1, CSN1S2, and CSN2) coding for these proteins.

An interesting observation was that the peaks of α_{s1}-CN and α_{s2}-CN, in the same individuals, were sometimes with a broad rather than a sharp shape, and of heights that fluctuated throughout the lactation/season. It is likely that these fluctuations could also be representing changes in the level of protein phosphorylation throughout the lactation/season, and quantification of phosphorylation throughout the season should be further investigated in individual sheep milk samples as studies are lacking in this area and these could be relevant for processability (Frederiksen et al. 2011; Ketto et al. 2017). It was noted that very few reports were available on the protein composition of sheep milk, and they were contradictory.

8.2.4 Genetic parameters of milk traits

Genetic studies were conducted to assess the extent of genetic variation for the various milk traits investigated, and to explore the correlations between them, with the aim of predicting indirect responses in traits that would not be directly targeted by selection due to feasibility reasons, which would be the case of processability traits, for example. Despite the small size of the dairy sheep flock studied, there was clear evidence of genetic variation in traits related to milk production, composition, and processability. This was reflected in the moderate heritability estimates, particularly for production traits, suggesting that selection pressure for milk production has not been strongly applied in this population. The moderate heritability indicates the potential for incorporating these traits into a selection index.

A favourable genetic correlation was found between milk yield traits and milk coagulation properties for cheese-making, suggesting that improving protein yield and fat yield—traits emphasized in New Zealand's dairy cattle selection index—could indirectly enhance milk quality for cheese-making in this dairy sheep flock. However, these correlations were not particularly strong. Additionally, an unfavourable correlation was observed between milk coagulation traits and milk heat stability, indicating that a different selection index may be

needed for dairy sheep flocks aimed at milk powder production, which is important for export revenue.

Traits that were more strongly genetically related to the processability of sheep milk included milk pH, the level of calcium, especially in relation to the casein content, the level of casein content in relation to the protein content, lactose content, and somatic cell count. These findings, supported by existing literature (Costa et al. 2019; Antanaitis et al. 2021), indicated that ewes' health status, including mammary gland health, could be related to the milk processability traits. Further studies could investigate more deeply the correlation between milk processability and ewe health, body condition, energy balance status, and heat stress, as they affect the metabolism and udder health, milk production and composition (Sevi and Caroprese 2012).

8.2.5 Genome-wide association analysis and genomic architecture of milk traits

Given the genetic variation found in the milk traits investigated, and the challenges in routine laboratory analysis of these processability traits on a commercial scale, it was important to explore the genetic control over these traits. This could potentially be implemented in animal breeding through genomic selection. The study identified significant SNPs and candidate genes across *Ovis aries* autosomes 2, 3, 6, 16, 18, 20, 25, and 26 associated with milk production, composition, and processability traits in dairy sheep. If the function of these genes is validated through gene expression studies, for example, they could be utilized in gene-assisted selection in dairy sheep breeding programs in the future.

Notable clusters (peaks) of SNP associations were observed across the Manhattan plots for most studied traits, evidencing genetic control in the peak regions, especially for contents of α_{s1}-CN (OAR6, OAR25, OAR 26), α_{s2}-CN (OAR6, OAR20), and α-lactalbumin (OAR3), with strong significant SNP associations at the Bonferroni significance level. Other milk traits investigated were only significantly associated with SNPs at a lower suggestive significance threshold. These traits included milk yield (OAR3), casein content (OAR3), ratio of casein to protein (OAR2), calcium content (OAR2), ratio of casein to calcium (OAR18), pH (OAR16), and rennet coagulation time (OAR16). Although only one SNP was significant for milk coagulation properties, and that was with rennet coagulation time, it was possible to see that the genetic architecture of the coagulation traits was quite similar to that of the ratio of casein to calcium, pH,

lactose, and the ratio of casein to protein, further supporting the findings from the genetic correlation estimates using pedigree information.

Another major finding was the gene PDZRN4, a candidate gene for milk yield that has also been recently associated by another research group with milk yield in New Zealand dairy sheep (Costilla et al. 2023), using three large flocks distinct from this one. This evidence points out that this gene's function should be further investigated in dairy sheep. This gene is involved in protein degradation pathways and has been associated with heat stress of dairy cows, which can cause a decrease in the expression of genes involved in milk production (Czech et al. 2023).

The most significant SNP for α_{s1}-CN, $-\log_{10}(P) = 6.09$, and α_{s2}-CN, $-\log_{10}(P) = 10.37$, was in gene BMP2K which is involved in protein phosphorylation. This finding is logical because the level of phosphorylation is related to the concentration of α_{s1}-CN and α_{s2}-CN. This finding could be of great importance for the technological and functional properties of sheep milk (Bijl et al. 2020). This gene's indirect role in phosphorylation of milk caseins should be further investigated. Other candidate genes reported in this study also had biological functions associated with protein phosphorylation (BMP2K, CCNT1, EPHA5, FBP1, GK2, LRRK2, MAPK10, NPFFR2, and TBK1) and should be further investigated.

8.3 Limitations of the study and recommendations for future research

A notable limitation of this study is the relatively small sample size for genetic and genomic analyses, as it was only feasible to include a single small flock of dairy sheep. This constraint was due to factors such as geographical location (of farms and laboratories), the number of people involved in the PhD project, associated costs, and limited industry interest. Consequently, the sample size may not fully represent the national genetic and environmental diversity across New Zealand's dairy sheep industry. However, it is worth noting that this farm has been selling ram and ewe lambs to other farms for several years, suggesting some genetic linkage between farms, particularly among smaller-scale operations. This potential connection, however, would need to be verified through further investigation. The contribution of this farm through participation in this project is thus of considerable significance to the sector.

Some characteristics of the farming system may represent a limitation to the study, such as the long lamb suckling period that did not allow for measurements in early lactation, only from mid-lactation onwards. Also, the shift from twice-a-day to once-a-day milking frequency in November, and this factor had to be included in the animal model of some chapters of the study.

Not all traits were included in GWAS due to the smaller dataset for them (ILCY and HCT). The milk coagulation properties are considered a standard way of measuring milk quality for cheese-making in the literature; however it could also be a generalizing interpretation of milk quality for cheese-making as cheeses are quite diverse products, with several manufacturing methods available for the various types of soft and hard cheese types. The laboratory cheese yield method might also be limiting due to lack of maturation step and small volume of sample.

The genome-wide association study was not effective in distinguishing genetic variants of the caseins or whey protein genes due to the lack of SNP coverage in these genes by the bead chip. Future studies could also consider performing SNP imputation to whole genome sequencing. This allows for prediction of unknown genotypes of animals genotyped at lower SNP density by using a reference set of animals genotyped at the higher SNP density.

Additional milk compositional traits that merit investigation alongside processability include the mineral profile of sheep milk, particularly the distribution of minerals between soluble and colloidal phases, which may contribute to milk pH. Other important factors to consider are the milk's buffering capacity and differential cell count. Additionally, exploring the genetic and genomic bases of traits influencing milk flavour, such as fatty acid composition, could be valuable as specific flavours could be limiting consumer acceptance of sheep milk. Moreover, continued research into the nutraceutical potential of sheep milk could enhance its market appeal and justify premium pricing.

It is recommended that the New Zealand dairy sheep industry defines a breeding goal for the development of a selection index. This will provide guidance for the estimation of economic values of traits and the effects of change in production, composition and processability traits on farm profit (Wolfová et al. 2009).

Future steps could involve expanding the use of genotyping and recording milk production and composition across additional flocks using a centralized data management platform.

With more data, a larger GWAS could be conducted. Alternatively, a meta-analysis combining GWAS results from this study and future ones in New Zealand would increase the likelihood of identifying true positives (Cantor et al. 2010), helping to narrow down the list of candidate genes influencing the milk production, composition, and processability traits directly or indirectly.

Subsequently, it would be valuable to identify genetically regulated genes from GWAS findings by conducting Transcriptome-Wide Association Studies (TWAS), to identify genes contributing to complex traits by linking genetic variation with transcriptional activity (Li and Ritchie 2021).

Another important next step would be to perform experimental validation of the target genes. This could involve full sequencing of the genes of interest followed by testing their impact on production, composition, and processability outcomes.

8.4 Conclusions

This study explored the genetic and genomic bases of milk production, composition, and processability in dairy sheep, and highlighted the need to continue genetic investigation within the developing dairy sheep industry in New Zealand. This thesis provides a groundwork that can be partially replicated in future research before the implementation of a selection index for dairy sheep. It was found potential to genetically improve milk production, composition, and processability in dairy sheep. Overall, improvement of milk production would lead to improvements in quality of milk in this flock for processing milk into cheese. However, this information requires further validation with a large recording scheme for wider applications.

It is possible that dairy sheep selection indexes be diversified to meet breeding objectives which will depend on the future payment systems in the sector. Theoretically, an example of this would be selection for cheese production versus milk processability for higher heat stability. There needs to be an investigation on additional traits that could be of economic importance for the sector other than milk traits, such as functional traits and animal's ability to maintain milk production (and milk composition and processability) in New Zealand environmental conditions. In grazing dairy systems, considerations should include not only milk quantity and quality but also factors like disease resistance, conformation, milking ability, and resilience, to reduce production costs, and factors to address sustainability concerns.

References

Antanaitis R, Juozaitienė V, Jonike V, Baumgartner W, Paulauskas A. 2021. Milk lactose as a biomarker of subclinical mastitis in dairy cows. Animals. 11: 1736.

Cantor RM, Lange K, Sinsheimer JS. 2010. Prioritizing GWAS results: a review of statistical methods and recommendations for their application. The American Journal of Human Genetics. 86: 6-22.

Costa A, Lopez-Villalobos N, Visentin G, De Marchi M, Cassandro M, Penasa M. 2019. Heritability and repeatability of milk lactose and its relationships with traditional milk traits, somatic cell score and freezing point in Holstein cows. Animal. 13: 909-916.

Frederiksen P, Andersen K, Hammershøj M, Poulsen H, Sørensen J, Bakman M, Qvist K, Larsen L. 2011. Composition and effect of blending of noncoagulating, poorly coagulating, and well-coagulating bovine milk from individual Danish Holstein cows. Journal of Dairy Science. 94: 4787-4799.

Johnson P, Newman S-A, McRae K, VanDerWeerden T, Brown M, Scobie D. 2022. A review of the current sheep industry in New Zealand and opportunities for change to meet future challenges.New Zealand Journal of Animal Science and Production 81: 1-15.

Ketto IA, Knutsen TM, Øyaas J, Heringstad B, Ådnøy T, Devold TG, Skeie SB. 2017. Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. International Dairy Journal. 70: 55-64.

Lees N, Lees I. 2018. Competitive advantage through responsible innovation in the New Zealand sheep dairy industry. International Food and Agribusiness Management Review 21: 505-524.

Li B , Ritchie MD. 2021. From GWAS to gene: transcriptome-wide association studies and other methods to functionally understand GWAS discoveries. Frontiers in genetics 12: 713230.

Raynal-Ljutovac K, Park Y, Gaucheron F, Bouhallab S. 2007. Heat stability and enzymatic modifications of goat and sheep milk. Small Ruminant Research. 68: 207-220.

Sevi A, Caroprese M. 2012. Impact of heat stress on milk production, immunity and udder health in sheep: A critical review. Small Ruminant Research. 107: 1-7.

Sevi A, Albenzio M, Marino R, Santillo A, Muscio A. 2004. Effects of lambing season and stage of lactation on ewe milk quality. Small Ruminant Research. 51: 251-259.

Trujillo A-J, Casals I, Guamis B. 2000. Analysis of major ovine milk proteins by reversed-phase high-performance liquid chromatography and flow injection analysis with electrospray ionization mass spectrometry. Journal of Chromatography A. 870: 371-380.

Wolfová M, Wolf J, Krupová Z, Kica J. 2009. Estimation of economic values for traits of dairy sheep: I. Model development. Journal of Dairy Science. 92: 2183-2194.

Supplementary information

“Udder and teat morphology traits associated with milk production and somatic cell score in dairy sheep from a New Zealand flock”

This chapter has been published elsewhere. It has been reformatted and presented here with permission:

Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2023. Udder and teat morphology traits associated with milk production and somatic cell score in dairy sheep from a New Zealand flock. New Zealand Journal of Agricultural Research Special issue: The New Zealand Society of Animal Production Annual Conference. https://doi.org/10.1080/00288233.2023.2248929

The findings of this chapter were presented orally in Session S2B: Young Members, at the New Zealand Society of Animal Production (NZSAP) Conference, held in conjunction with the New Zealand Grassland Association Conference in Rotorua, New Zealand, 14-16 November, 2023.

Abstract

The objective of this study was to estimate the phenotypic correlations between udder and teat morphology traits, milk production traits, and somatic cell score in dairy sheep from a flock in New Zealand. A total of 162 lactating ewes were scored for morphology traits during the milk production season of 2021-2022. The 130-d lactation yields of milk, fat, protein, and lactose were obtained with 2-4 test-days from each ewe and modelled using random regression with orthogonal polynomials. Age had a significant effect on all udder and teat traits. Coat colour (genetic variety within the breed; white or black) was a significant effect for teat angle and udder separation. Udders that were above the hock were associated with lower milk, fat, protein, and lactose yields. Udders with well-defined separation between halves were associated with higher milk, protein, and lactose yields, and with lower somatic cell count. Well-attached udders were associated with lower fat yield and lower somatic cell score. Teats with a backwards angle were associated with lower milk and lactose yields. Further studies are needed to estimate heritability and genetic correlations between these traits to determine whether these traits should be implemented in breeding programs for dairy sheep in New Zealand.

Keywords

Dairy sheep; Udder morphology traits; Teat morphology traits; Milk production; Somatic cell score.

S.1 Introduction

The New Zealand dairy sheep industry is relatively new. This sector started in 1992 with the importation of a small pool of East Friesian genetics (Lees and Lees 2016) that were crossed with other breeds. More recently, Awassii and Lacaune have also been incorporated in some New Zealand flocks (Ministry of Primary Industries and Massey University 2020, Peterson and Prichard 2015). Although small, this industry is growing due to the high nutritional value of sheep milk seen by the higher concentrations of proteins, fats, vitamins, and minerals when compared to cow and goat milks (Park et al. 2007), and due to the potential to be used as functional food and promote human health (Mohapatra et al. 2019). There is also potentially lower environmental impact caused by dairy sheep farming when compared to the traditional dairy cow farming (Downie-Melrose 2014; Smith et al. 2018).

In New Zealand, as in many other countries, dairy animals (including sheep, cows, goats, deer) are milked in parlours with machinery that uses mechanical pulsation and clusters (cups) that attach to the teats. Poor mammary morphology is mainly characterised by long pendulous udders and by teats positioned too laterally or too vertically, which is not desirable for cluster attachment during milking or for suckling lambs, respectively. Therefore, mammary morphology is crucial at the farm and can affect the everyday efficiency of milking and of nursing of lambs. Also, udder and teat traits can have moderate to high heritability, depending on the breed, and these traits have been included in the selection index of some dairy sheep populations around the world (Fernández et al. 1997; Legarra and Ugarte 2005; Casu et al. 2006).

Furthermore, it has been reported that selection of dairy sheep solely for milk yield can indirectly result in impaired udder morphology for machine milking and lamb suckling (Legarra and Ugarte 2005; Marie-Etancelin et al. 2005), reduced milk solids (Bencini 2002), increased somatic cell count (Fernández et al. 1997; Legarra and Ugarte 2005), and reduced fertility (David et al. 2008).

There is limited information available in the scientific literature about genetic programmes for dairy sheep in New Zealand. One study using in a single flock has proposed a model for genetic evaluation of dairy sheep in New Zealand (Scholtens et al. 2018), without considering udder and teat morphology traits. However, most farms still rely on their own selection schemes. More recently, large New Zealand dairy sheep companies have started running their own genetic programmes claiming to have a breeding goal and a selection index that includes udder conformation traits (New Zealand Sheepbreeders Association 2023a,b) alongside milk yield, and milk composition traits. Nationally, there is still not one defined breeding goal for dairy sheep.

Only a few studies have investigated udder traits in New Zealand sheep, and these were done on non-dairy sheep, where udder depth was phenotypically associated with lamb survival to weaning (Griffiths et al. 2019), and lamb weight and milk volume (Yusuf et al. 2018). The aim of this study was to investigate the phenotypic correlations between udder and teat morphology traits, milk production traits and somatic cell score (SCS) in dairy sheep from a New Zealand flock. Knowledge of the relationship between udder morphology traits and milk production is valuable to inform the New Zealand dairy sheep industry for further genetic studies on these traits, using larger populations of dairy sheep.

S.2 Materials and methods

Test-day records were gathered during the milk production season of 2021-2022, from 162 ewes from a commercial farm, in Masterton, New Zealand. An animal ethics approval was obtained for this study (Massey University Animal Ethics Committee-Protocol 21/45). The breed used in the current study is mainly formed by East-Friesian, with some Coopworth and Border Leicester bloodlines. In this flock, ewes can distinctly be separated by coat colour, black and white.

The flock was milked twice-a-day (TAD) in October. In November, milking frequency was shifted to once-a-day (OAD) to align with the seasonal availability of pasture, this is a common practice on this farm. Therefore, ewes that had their lambs weaned after 31 Oct 2021 were only milked once-a-day. To adjust for the milking frequency, days in TAD milking were calculated for each ewe (1st of November−weaning date).

Milk yield was recorded on the test-day, and a representative milk sample was taken for composition analyses, as described in Marshall et al. (2023). The lactation curves were modelled using 2-4 test-day records from each ewe. The 130-day lactation yields of milk, fat, protein, and lactose were obtained using random regression with orthogonal polynomials (Marshall et al. 2023). Time was defined as d = days in milk−35, as most records were made 35 days after the lambing date due to the exclusive suckling period. Legendre polynomials were used to standardise values to the interval [-1, …., 1], and the coefficients were then calculated using the Rodrigues formula (Askey 2005):

$P0(t) = 1,$

$P1(t) = x,$

$P2(t) = \frac{1}{2}(3x^2 - 1),$

$P3(t) = \frac{1}{2}(5x^3 - 3x),$

$P4(t) = \frac{1}{8}(35x^4 - 30x^2 + 3),$

$P5(t) = \frac{1}{8}(63x^5 - 70x^3 + 15x)$

where $x = -1 + 2$, and $\frac{(t - tmin)}{(tmax - tmin)}$, with tmin = 1 and tmax = 130.

Day 1 corresponded to day 35 of lactation.

The random regression model was represented as follows:

$$y_{ti} = (\beta_0 P_0 + \beta_1 P_{1t} + \beta_2 P_{2t} + \cdots + \beta_n P_{nt}) + (\alpha_{0i} P_0 + \alpha_{1i} P_1 + \alpha_{2i} P_2 + \cdots + \alpha_{ni} P_n) + e_{ti},$$

where y_{ti} is the observed yield of animal i at day t, β's are the fixed regression coefficients of the lactation curve of the population, α values are random regression coefficients describing the lactation curve for animal i, n is the maximum polynomial order, and e_{ti} is the random residual of observation y_{ti}. The estimates of β and α were obtained using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) with the COVTEST option for covariance parameter estimates. Based on the Akaike (AIC) and Bayesian (BIC) information criteria (smallest is the best), an orthogonal polynomial of order 4 was considered the best fit for modelling daily milk and lactose yields. An orthogonal polynomial of order 5 was considered the best fit for modelling fat and protein daily yields.

The daily milk yield was predicted from 35 to 164 days in milk (or from t = 1 to t = 130) and were then summed to obtain an estimated total milk yield produced by each ewe for 130 days (from day 35 to day 164 of lactation).

Somatic cell score for each ewe was averaged from the somatic cell score obtained from each test-day, calculated as SCS = Log_2(somatic cell count). Throughout the current study, no ewes presented any clinical signs of udder or teat inflammation.

Udder and teat morphology were visually assessed on the 162 lactating ewes once in the season, in December 2021. At the time of assessment, the ewes were in mid to late lactation stages and were being milked once-a-day, in the afternoons. Udder and teat assessments were done immediately prior to milk collection. Selection on teat and udder morphology traits and on milk production traits has been in place for over 7 years in this farm. High repeatability for teat and udder morphology traits within a season (or lactation) has been reported for other breeds around the world, such as Sarda (Casu et al. 2006), Lacaune (Marie-Etancelin et al. 2003), and Churra (De la Fuente et al. 1996), meaning that a single score in the season should be reliable. Assessment of most teat and udder morphology traits was performed from behind the animals as they stood in their bays in the milking shed, except for teat angle, which was performed laterally. These traits were linear scores

from 1 to 9 (Figure S1) and include teat position (TP), teat angle (TA), udder depth (UD), udder attachment (UA), and udder separation (US). The desirable value for some udder traits is, in some cases, the highest score or the intermediate score. For udder depth, for example, given its positive relationship with milk production an intermediate score is preferable (Caja et al. 2000).

As there is no single international scoring system in place for sheep udder and teat traits, this study has defined them based on a combination of guidelines from the International Committee for Animal Recording (ICAR 2018) for the different breeds of dairy sheep, and from previous publications for dairy goats (McLaren et al. 2016) and dairy sheep (Casu et al. 2006; Griffiths et al. 2019), whilst aiming similarity with the scoring system that is already in place for dairy cows in New Zealand (Advisory Committee on Traits Other than Production, 2020).

Descriptive statistics (mean, standard deviation, and coefficient of variation) for traits were obtained in SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). Analysis of variances for the dependent variables udder and teat morphology traits, milk production traits (milk yield, fat yield, protein yield, lactose yield), and SCS were performed in SAS using the MIXED procedure with a linear model that included the fixed effects of ewe coat colour as an indicator of genetic variety within the breed (categorical variable with two levels: black or white ewe), litter size (categorical variable with two levels: 1 lamb or 2 lambs and greater), ewe age (categorical variable with four levels: 1, 2, 3, and 4 years and older), and days in twice-a-day (TAD) milking as covariate, and random residual error. Least squares means and standard errors for each class of the fixed effects were obtained and used for mean comparisons using Fisher's least significant difference.

Using the solutions of the mixed model, best linear prediction functions were used to predict values of dependent traits at different days milked TAD after weaning.

Partial correlation coefficients between traits were obtained through multiple analysis of variance, using the option MANOVA of the GLM procedure with the same linear model described above. These partial correlation coefficients can be considered as phenotypic correlations because these coefficients are adjusted by fixed effects included in the linear model.

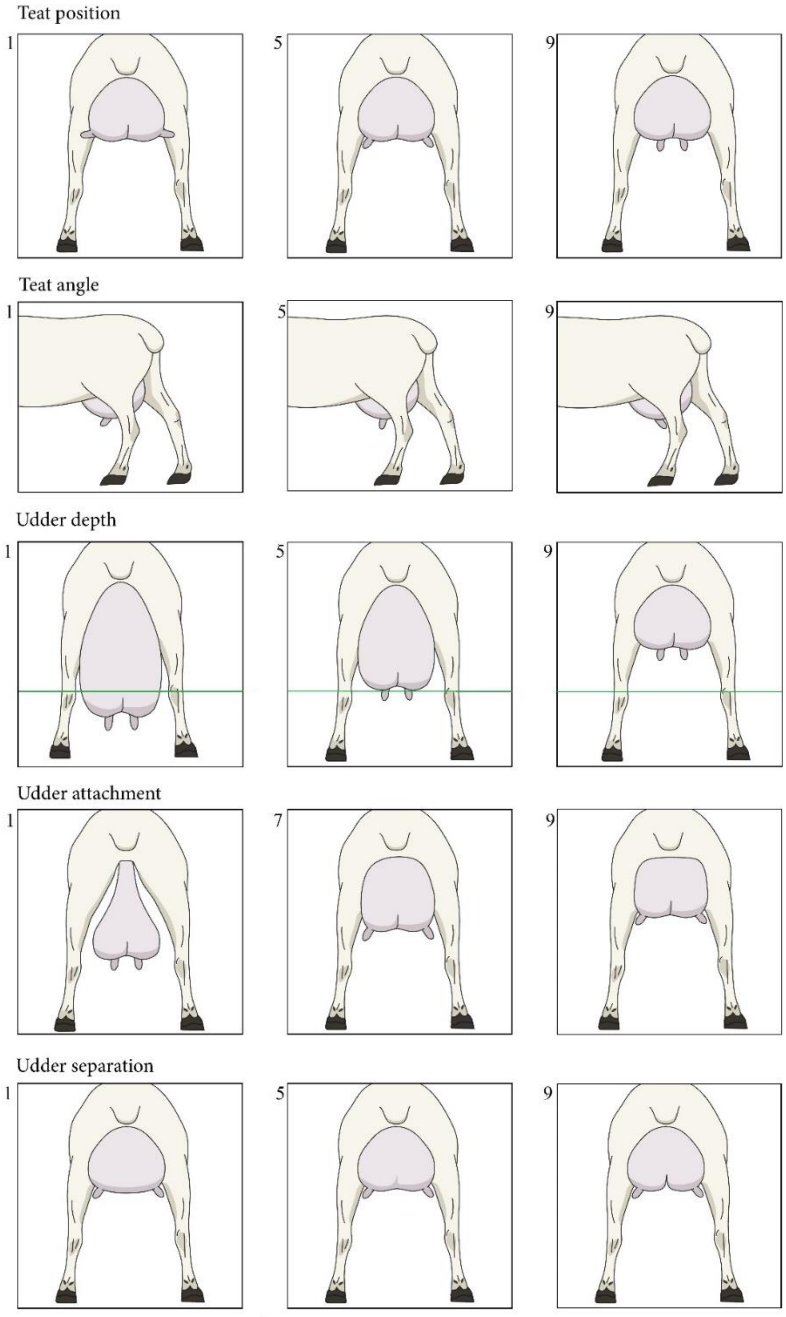


Figure S1. Linear scoring system of udder and teat morphology traits used in ewes from a commercial flock during the production season of 2021-2022. Teat position= the distance between the teats and the lowest part of the udder (1= horizontal; 9= vertical). Teat angle= looking from the side of the animal (1=pointing forward; 9= pointing towards the rear). Udder depth= the distance between the udder cleft and the abdominal wall, taking as reference point the line joining the hocks (1= deep; 5= hock level; 9= shallow). Udder attachment= the ratio between udder height and udder width (1= width smaller than height; 7= width equals height, or square; 9= width larger than height). Udder cleft or udder separation= degree of separation between left and right mammary glands (1= separation is missing; 5=average separation; 9= well marked separation.

S.3 Results

Average milk production per ewe estimated for 130 days of milking, and average udder and teats scores are presented on Table S1. Coefficients of variation for milk production traits were high and ranged from 27 to 31%, and for teat and udder morphology traits ranged from 11 to 25%, being the lowest variation for udder depth (11%) and teat angle (13%).

The F- and *p*-values for fixed effects are also presented in Table S1. Age and days in TAD milking significantly affected yields of milk, fat, protein, and lactose. The factor that had the greatest effect on the total yields was days in TAD milking (largest F-values). Litter size and coat colour did not have any significant effect on milk production. SCS was not affected by any of the fixed effects.

All teat and udder morphological traits were strongly affected by age ($p<0.001$), especially udder attachment and udder depth, with F values of 22.89 and 10.15, respectively. Coat colour significantly affected teat angle ($p<0.001$) and udder separation ($p<0.01$). Litter size and days in TAD milking did not significantly affect any of the teat and udder morphology traits (Table S1).

Least squares means for total lactation yields of milk, fat, protein, and lactose and least squares means for teat and udder morphology traits are presented in Table S2. One-year-old ewes produced significantly less ($p<0.05$) milk, fat, protein, and lactose than older ewes (Table S2). Three-year-old ewes produced the highest yields of milk, protein, and lactose, whereas four-year-old ewes produced the highest yield of fat. Ewes that lambed late in relation to the median lambing date of the flock missed TAD milking in early lactation (days in TAD = 0) and produced significantly less ($p<0.05$) milk, fat, protein, and lactose yield than early lambing ewes that were milked TAD in early lactation (days in TAD $\geq$ 14). Four-year-old ewes and older ewes had significantly lower ($p<0.05$) scores for udder attachment and udder separation.

Phenotypic correlations (r_P) between milk production, SCS, and udder and teat morphology traits are presented on Table S3. Correlations among milk production traits (milk, fat, protein, and lactose yields) were significant ($p<0.001$), positive, and high ($r_P>0.9$). Correlations among all teat and udder traits were also positive, these correlations were significant ($p<0.001$), and moderate between most teat and udder morphology traits, ranging from 0.31 to 0.61 (Table S3).

All teat and udder morphology traits, except for udder separation, had negative correlations with milk production traits (milk, fat, protein and lactose yields) (Table S3). The correlations between udder depth and milk production traits were all significant ($p<0.001$) and moderate, ranging from -0.34 to -0.36. Other correlations between udder/teat morphology and milk production traits were weak and/or non-significant, ranging from -0.17 to 0.20.

Somatic cell score had a negative correlation with teat angle, udder depth, udder attachment, and udder separation. The correlation between SCS and udder attachment was significant ($p<0.01$) and moderate (-0.24), and the correlation with udder separation was also significant ($p<0.05$), but low (-0.18).

Table S1. Descriptive statistics[1] and F-values for factors affecting milk production (estimated lactation yields of milk, fat, protein, and lactose) and udder and teat morphology traits in dairy sheep from a New Zealand flock during the milk production season of 2021-2022 (N = 162).

Trait	Mean	SD	Min	Max	CV (%)	F-value			
						Coat colour	Age	Litter size	Days in TAD[3]
Lactation yields[2] (kg)									
Milk	86.1	26.1	25.1	168.8	30	0.07	6.70 ***	0.63	19.86 ***
Fat	5.1	1.4	2.0	9.5	28	0.61	3.83 **	0.00	27.47 ***
Protein	4.5	1.2	1.7	8.2	27	0.01	4.38 **	1.12	20.51 ***
Lactose	4.1	1.3	1.2	8.0	31	0.03	6.71 ***	0.63	17.48 ***
Average SCS	16.9	1.7	14.1	23.0	10	0.94	1.70	1.48	0.79
Teat morphology									
Teat angle	5.5	0.7	4	7	13	12.22***	6.82***	2.76	0.09
Teat position	5.6	1.4	1	9	25	2.98	6.47***	0.03	2.87
Udder morphology									
Udder depth	7.6	0.8	5	9	11	0.45	10.15***	0.90	0.47
Udder attachment	7.1	1.6	2	9	23	0.00	22.89***	0.88	1.83
Udder separation	6.7	1.6	1	9	24	5.26**	6.96***	0.10	0.73

[1]SD = standard deviation, Min = minimum value, Max = maximum value, CV = coefficient of variation.

[2]Lactation yields estimated from daily yields from 35 to 164 days of lactation; Average SCS = average of somatic cell scores (SCS) during the lactation where SCS = Log_2(somatic cell count); Teat and udder morphology traits were scored from 1 to 9.

[3]Days in twice-a-day milking.

Statistical significance is given as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S2. Least-squares means ± standard errors of milk production traits (estimated lactation yields of milk, fat, protein, and lactose) and udder and teat morphology scores for different ewe ages (year), litter sizes, coat colour, and days in twice-a-day (TAD) milking, in dairy sheep from a New Zealand flock during the milk production season of 2021-2022.

Effect	N	Milk	Fat	Protein	Lactose	TA	TP	UD	UA	US
Age										
1	23	$64.3^{b} \pm 5.4$	$4.1^{b} \pm 0.30$	$3.6^{b} \pm 0.30$	$3.1^{b} \pm 0.30$	$5.86^{a} \pm 0.54$	$6.30^{a} \pm 0.97$	$8.26^{a} \pm 0.61$	$8.21^{a} \pm 0.59$	$7.26^{a} \pm 1.35$
2	46	$85.8^{a} \pm 3.6$	$5.0^{a} \pm 0.20$	$4.5^{a} \pm 0.17$	$4.2^{a} \pm 0.18$	$5.84^{a} \pm 0.55$	$5.97^{a} \pm 1.12$	$7.89^{a} \pm 0.67$	$7.67^{a} \pm 0.81$	$7.02^{a} \pm 1.30$
3	38	$92.1^{a} \pm 3.6$	$5.2^{a} \pm 0.20$	$4.7^{a} \pm 0.17$	$4.4^{a} \pm 0.18$	$5.44^{b} \pm 0.55$	$5.52^{b} \pm 1.13$	$7.65^{b} \pm 0.74$	$7.50^{a} \pm 1.10$	$6.97^{a} \pm 1.30$
≥ 4	55	$90.2^{a} \pm 3.0$	$5.3^{a} \pm 0.17$	$4.6^{a} \pm 0.14$	$4.3^{a} \pm 0.15$	$5.21^{b} \pm 0.83$	$5.10^{b} \pm 1.79$	$7.16^{c} \pm 0.87$	$5.78^{b} \pm 1.96$	$5.98^{b} \pm 1.85$
Litter size										
1	64	81.7 ± 2.8	4.9 ± 0.16	4.3 ± 0.14	3.9 ± 0.14	5.48 ± 0.77	5.81 ± 1.43	7.82 ± 0.82	7.43 ± 1.45	6.92 ± 1.51
≥2	98	84.5 ± 2.9	4.9 ± 0.16	4.4 ± 0.14	4.1 ± 0.14	5.58 ± 0.67	5.50 ± 1.42	7.52 ± 0.84	6.82 ± 1.72	6.54 ± 1.63
Coat colour										
Black	32	82.5 ± 4.0	4.8 ± 0.22	4.3 ± 0.20	3.9 ± 0.20	$5.03^{b} \pm 0.82$	5.71 ± 1.32	7.40 ± 0.97	6.68 ± 1.57	$7.03^{a} \pm 1.33$
White	130	83.7 ± 1.9	5.0 ± 0.11	4.4 ± 0.09	4.0 ± 0.10	$5.66^{a} \pm 0.62$	5.60 ± 1.46	7.70 ± 0.80	7.16 ± 1.66	$6.60^{b} \pm 1.64$
Days in TAD										
0	53	$71.2^{b} \pm 3.2$	$4.1^{b} \pm 0.18$	$3.8^{c} \pm 0.15$	$3.4^{b} \pm 0.16$	5.60 ± 0.71	5.50 ± 1.46	7.69 ± 1.04	7.07 ± 1.94	6.67 ± 1.64
14	36	$86.8^{a} \pm 4.0$	$5.1^{a} \pm 0.22$	$4.5^{b} \pm 0.19$	$4.2^{a} \pm 0.20$	5.66 ± 0.67	6.02 ± 1.44	7.66 ± 0.75	7.16 ± 1.27	7.00 ± 1.47
21	37	$86.3^{a} \pm 4.0$	$5.3^{a} \pm 0.22$	$4.4^{b} \pm 0.19$	$4.2^{a} \pm 0.20$	5.32 ± 0.78	5.59 ± 1.40	7.75 ± 0.72	7.40 ± 1.16	6.67 ± 1.52
28	36	$94.8^{a} \pm 4.2$	$5.6^{a} \pm 0.24$	$5.0^{a} \pm 0.20$	$4.5^{a} \pm 0.21$	5.55 ± 0.65	5.41 ± 1.38	7.41 ± 0.69	6.61 ± 1.87	6.41 ± 1.69

N = number of ewes within each category. Days in TAD = 0 (ewes that were only milked once-a-day). Days in TAD = 14 (ewes that were milked for 14 days in twice-a-day milking). Days in TAD = 21 (ewes that were milked for 21 days in twice-a-day milking). Days in TAD = 28 (ewes that were milked for 28 days in twice-a-day milking). ^{a,b,c} Means with different superscripts within effect are significantly different ($p < 0.05$).

Table S3. Phenotypic correlations between estimated lactation yields (milk, fat, protein, and lactose), SCS, and udder and teat morphology traits in dairy sheep from a New Zealand flock during the milk production season of 2021-2022.

Trait[1]	Milk	Fat	Protein	Lactose	SCS	Teat angle	Teat position	Udder depth	Udder attachment
Fat	0.92***								
Protein	0.97***	0.92***							
Lactose	0.99***	0.91***	0.96***						
SCS	-0.09	-0.13	-0.05	-0.13					
Teat angle	-0.16*	-0.15	-0.14	-0.17*	-0.04				
Teat position	-0.05	-0.09	-0.02	-0.05	0.01	0.35***			
Udder depth	-0.35***	-0.36***	-0.35***	-0.34***	-0.09	0.36***	0.12		
Udder attachment	-0.12	-0.17*	-0.12	-0.12	-0.24**	0.31***	0.32***	0.61***	
Udder separation	0.19*	0.12	0.19*	0.20*	-0.18*	0.05	0.37***	0.14	0.34***

[1]Milk, fat, protein, and lactose are lactation yields estimated from daily yields from 35 to 164 days of lactation; SCS is the average of somatic cell scores (SCS) during the lactation where SCS = Log_2(somatic cell count); Teat and udder morphology traits were scored from 1 to 9.

Statistical significance is given as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

S.4 Discussion

Udder depth was the main morphological trait correlated with all milk production traits, with significant ($p < 0.001$) moderate phenotypic correlations with yields of milk, fat, protein, and lactose (r_P ranging from -0.34 to -0.36). Higher udders were related to lower milk production, with age included as one of the fixed effects. This study agrees with the negative phenotypic relationship between udder depth and milk production reported in various breeds of dairy sheep (Casu et al. 2000; Kominakis et al. 2009; Pourlis 2020; Vrdoljak et al. 2020). In this study, udder depth scores only ranged from 5 to 9, meaning that no ewe had very low udders (below the hock line). This could be a consequence of selection in this flock for udder depth. Intermediate scores for udder depth would be desirable for both lamb suckling and machine milking (cluster attachment) without compromising milk productivity. All other teat and udder morphological traits only had low phenotypic correlations ($r_P \leq 0.2$) with the milk production traits.

High level of udder attachment for machine milking was associated with lower SCS. High level of udder attachment reflects the better support of the udder given by the lateral suspensory ligaments. Udders that are not pendulous (with less movement) and that are far off the ground, are less prone to damage (bruising and laceration) and are less prone to contamination in the teat canal, preventing infection of the mammary glands, reducing the occurrence of subclinical mastitis. Udder depth and udder attachment were highly correlated traits ($r_P = 0.61$), so it would be preferable to use udder attachment as it is a more complete type of assessment that includes width and height. Also, variation for udder attachment was higher than variation for udder depth in this flock, which could be translated into more opportunity for selection on udder attachment, but further studies are needed to confirm animal genetic variance for these traits.

Udder attachment also had a low negative correlation fat yield ($r_P = -0.17$), agreeing with the findings of McKusick et al. (1999) for East Friesian crossbred ewes, where for each cm increase in cistern height there was a relative increase of 0.12% units of milk fat. Ewes with deeper cisterns can store milk and milk fat in the cistern between milkings and avoid the deleterious effects of residual milk on the secretory alveoli of the udder (McKusick et al. 1999).

Udders with good separation between halves (groove between mammary glands) also had a weak but significant ($p < 0.05$) negative correlation with SCS, agreeing with others

(Fernández et al. 1997; Legarra and Ugarte 2005). Good udder separation is a reflect of better support given by the medial suspensory ligament (Casu et al. 2006). Udders with well-defined separation had a low positive correlation ($r_P \leq 0.20$) with the milk production traits. These correlations were significant ($p < 0.05$) with milk, protein, and lactose yields.

All udder and teat morphology traits were positively correlated amongst themselves agreeing with the phenotypic correlation results reported by others (Fernández et al. 1995, Kominakis et al. 2009). Further studies are needed to investigate the genetic correlations between udder and teat morphology traits to confirm whether improvement in one of the teat and udder morphological traits would also result in improvement of the other traits. Also, a principal component analysis would indicate the group of teat and udder morphological traits that largely explain variance in the dataset.

Age strongly affected all udder and teat conformation traits, especially udder attachment and udder depth, given the high F values (Table S1), agreeing with Serrano et al. (2002). Low udders, of poor attachment, with poor separation, with lateral teats, and with teats pointing forward were more commonly observed after the third parity (Table S2). High scores for udder and teat conformation traits were more commonly observed on ewes of first and second parities. This is expected, as worsening of the teat and udder conformation traits with the parities has been reported (Casu et al. 2006). To confirm repeatability of overall udder conformation with ageing, principal component analysis across seasons would be useful.

This study confirms that teat and udder morphology scores have negative phenotypic association with milk production. However, intermediate scores for teat and udder conformation traits were good enough for machine milking and for acceptable levels of SCS, without compromising milk production. It is recommended that dairy sheep farmers implement a recording system of udder and teat conformation scores, as well as accurate recording of test-day milk yield and milk composition for the purposes of genetic selection. In addition to this, the New Zealand dairy sheep industry should consider a central database for performance recording of dairy sheep. This would enable nation-wide genetic evaluation on these traits and provide genetic information for commercial farmers.

S.5 Conclusion

This was the first study to describe teat and udder traits in dairy sheep in a commercial flock in New Zealand. The results from the multivariate analysis indicate that udder and teat conformations that are highly desirable for machine milking are correlated with lower milk production. Therefore, the selection of animals on machine milking traits should be carefully planned, so that milk yield is not compromised. However, further studies are needed to define heritability for teat and udder morphology traits, and genetic correlations between teat and udder morphology traits with milk production traits, for possible implementation in selection schemes for dairy sheep in New Zealand.

References

Advisory Committee on Traits Other than Production. 2020. Evaluation system for traits other than production for dairy cattle in New Zealand.

Askey R. 2005. The 1839 paper on permutations: Its relation to the Rodrigues formula and further developments. In Mathematics and Social Utopias in France: Olinde Rodrigues and His Times, History of Mathematics; Altmann, S., L., Ortiz, E.L., Eds.; American Mathematical Society: Providence, RI, USA, 2005; Volume 28, pp. 105–118, ISBN 978-0-8218-3860-0.

Bencini R. 2002. Factors affecting the clotting properties of sheep milk. Journal of the Science of Food and Agriculture. 82: 705-719.

Caja G, Such X, Rovai M. 2000. Udder morphology and machine milking ability in dairy sheep. Dairy Sheep Symposium Pg. 17-40.

Casu S, Deiana S, Tolu S, Carta A. 2000. Linear evaluation of udder morphology in Sarda dairy sheep: Relationship with milk yield. Proc. Atti VIX Cong. Nat., SIPAOC 1: 195-198.

Casu S, Pernazza I, Carta A. 2006. Feasibility of a linear scoring method of udder morphology for the selection scheme of Sardinian sheep. Journal of Dairy Science. 89: 2200-2209.

David I, Astruc J-M, Lagriffoul G, Manfredi E, Robert-Granié C, Bodin L. 2008. Genetic correlation between female fertility and milk yield in Lacaune sheep. Journal of Dairy Science. 91: 4047-4052.

De la Fuente LF, Fernandez G, San Primitivo F. 1996. A linear evaluation system for udder traits of dairy ewes. Livestock Production Science. 45: 171–178.

Downie-Melrose K. 2014. A Bio-economic feasibility study of sheep dairy systems in Canterbury. Lincoln University, Lincoln, New Zealand.

Fernández G, Alvarez P, San Primitivo F, De la Fuente LF. 1995. Factors Affecting Variation of Udder Traits of Dairy Ewes. Journal of Dairy Science. 78: 842-849.

Fernández G, Baro J, De la Fuente L, San Primitivo F. 1997. Genetic parameters for linear udder traits of dairy ewes. Journal of Dairy Science. 80: 601-605.

Griffiths KJ, Ridler AL, Compton C, Corner-Thomas RA, Kenyon PR. 2019. Investigating associations between lamb survival to weaning and dam udder and teat scores. New Zealand Veterinary Journal. 67: 163-171.

ICAR. 2018. Section 16- Guidelines for performance recording in dairy sheep and dairy goats: 1-37.

Kominakis A, Papavasiliou D, Rogdakis E. 2009. Relationships among udder characteristics, milk yield and, non-yield traits in Frizarta dairy sheep. Small Ruminant Research. 84: 82-88.

Lees I, Lees NJ. 2016. Competitive advantage through responsible innovation in the New Zealand's sheep dairy industry. International Food and Agribusiness Management Review 21. doi 10.22434/IFAMR2017.0013

Legarra A, Ugarte E. 2005. Genetic Parameters of Udder Traits, Somatic Cell Score, and Milk Yield in Latxa Sheep. Journal of Dairy Science. 88: 2238-2245.

Mohapatra A, Shinde AK, Singh R. 2019. Sheep milk: a pertinent functional food. Small Ruminant Research. 181: 6-11.

Marie-Etancelin C, Astruc J-M, Porte D, Larroque H, Robert-Granié C. 2005. Multiple-trait genetic parameters and genetic evaluation of udder-type traits in Lacaune dairy ewes. Livestock Production Science. 97: 211-218.

Marie-Etancelin C, Aurel MR, Barillet F, Jacquin M, Pailler F, Porte D, Casu S, Carta A, Deiana S, Tolu S. 2003. New tools to appraise udder morphology and milkability in dairy sheep. CIHEAM, Options Méditerranéennes : Série A. Séminaires Méditerranéens 55: 71-79.

Marshall AC, Lopez-Villalobos N, Loveday SM, Ellis A, McNabb W. 2023. Modelling Lactation Curves for Dairy Sheep in a New Zealand Flock. Animals. 13: 349.

McKusick BC, Berger YM, Thomas DL. 1999. Preliminary results: effects of udder morphology on commercial milk production of East friesian crossbreed ewes. Proceedings of the 5th Great Lakes Dairy Sheep Symposium, Brattleboro, Vermont, USA.

McLaren A, Mucha S, Mrode R, Coffey M, Conington J. 2016. Genetic parameters of linear conformation type traits and their relationship with milk yield throughout lactation in mixed-breed dairy goats. Journal of Dairy Science. 99: 5516-5525.

Ministry of Primary Industries and Massey University. 2020. New Zealand Sheep Dairy Survey, Wellington and Palmerston North: MPI-Massey University. https://www.mpi.govt.nz/dmsdocument/43183-Sheep-Dairy-Survey-Report-July-2020. [accessed on 04 May 2024].

New Zealand Sheepbreeders Association. 2023a. https://nzsheep.co.nz/zealandia/. [accessed on 18 Juy 2023].

New Zealand Sheepbreeders Association. 2023b. https://nzsheep.co.nz/southern-cross/ [accessed on 18 Juy 2023].

Park Y, Juárez M, Ramos M, Haenlein G. 2007. Physico-chemical characteristics of goat and sheep milk. Small Ruminant Research. 68: 88-113.

Peterson SW, Prichard C. 2015. The sheep dairy industry in New Zealand: a review. Proceedings of the New Zealand Society of Animal Production 75: 119-126.

Pourlis A. 2020. Ovine mammary morphology and associations with milk production, milkability and animal selection. Small Ruminant Research. 184: 106009.

SAS. Statistical Analysis System, Version 9.4; SAS Institute Inc.: Cary, NC, USA, 2004.

Scholtens MR, Peterson S, Gunson K, Gunson A, Lopez-Villalobos N. 2018. Genetic evaluation of dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. 61: 81-91.

Serrano M, Perez-Guzman M, Montoro V, Jurado J. 2002. Genetic analysis of udder traits in Manchega ewes. Livestock Production Science. 77: 355-361.

Smith L, Longhurst R, Monaghan R, Bartlett N. 2018. Characterising the volumes and chemical characteristics of dairy sheep effluent in New Zealand. New Zealand Journal of Agricultural Research. 61: 109-117.

Vrdoljak J, Prpić Z, Samaržija D, Vnučec I, Konjačić M, Kelava Ugarković N. 2020. Udder morphology, milk production and udder health in small ruminants. Mljekarstvo: Časopis Za Unaprjeđenje Proizvodnje I Prerade Mlijeka 70: 75-84.

Yusuf O, Logan CM, Ridler AL, Greer AW. 2018. Investigation into udder characteristics, mastitis, and milk production in crossbred sheep. New Zealand Journal of Animal Science and Production. 78: 82-87.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Ana Carolina Marshall
Name and title of main supervisor:	Prof. Nicolas Lopez-Villalobos
In which chapter is the manuscript/published work?	Chapter 3

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Conceptualisation, A.C.M, N.L.-V. and W.M.; methodology, A.C.M. and N.L.-V.; software, N.L.-V.; validation, N.L.-V.; formal analysis, A.C.M.; investigation, A.C.M.; resources, N.L.-V., S.M.L., A.E., and W.M.; data curation, A.C.M.; writing—original draft preparation, A.C.M.; writing—review and editing, A.C.M., N.L.-V., S.M.L., A.E., and W.M.; visualisation, A.C.M.; supervision, N.L.-V., S.M.L., A.E., and W.M.; project administration, A.C.M.; funding acquisition, W.M. All authors have read and agreed to the published version of the manuscript.

Please select one of the following three options:

- (●) **The manuscript/published work is published or in press**

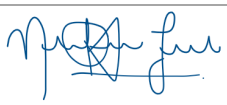
  Please provide the full reference of the research output:

  Marshall AC, Lopez-Villalobos N, Loveday SM, Ellis A, McNabb W. 2023. Modelling lactation curves for dairy sheep in a New Zealand flock. Animals. 13: 349. https://doi.org/10.3390/ani13030349

- ( ) **The manuscript is currently under review for publication**

  Please provide the name of the journal:

- ( ) **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:		Main supervisor's signature:	

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Ana Carolina Marshall
Name and title of main supervisor:	Prof. Nicolas Lopez-Villalobos
In which chapter is the manuscript/published work?	Chapter 4

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Ana Carolina Marshall: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Nicolas Lopez-Villalobos: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Conceptualization. Simon M. Loveday: Writing – review & editing, Supervision. Mike Weeks: Writing – review & editing, Supervision. Warren McNabb: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Please select one of the following three options:

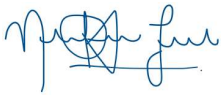
- (●) **The manuscript/published work is published or in press**

  Please provide the full reference of the research output:
  Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024. Animal factors affecting the cheese-making properties and the heat coagulation time of milk from dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. Published online: 27 Mar 2024. https://doi.org/10.1080/00288233.2024.2333826

- ( ) **The manuscript is currently under review for publication**

  Please provide the name of the journal:

- ( ) **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:		Main supervisor's signature:	

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Ana Carolina Marshall
Name and title of main supervisor:	Prof. Nicolas Lopez-Villalobos
In which chapter is the manuscript/published work?	Chapter 5

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Ana Carolina Marshall: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Vania Vigolo: Methodology. Massimo De Marchi: Writing – review & editing, Methodology. Nicolas Lopez-Villalobos: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Conceptualization. Simon M. Loveday: Writing – review & editing, Supervision. Mike Weeks: Writing – review & editing, Supervision. Warren McNabb: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Please select one of the following three options:

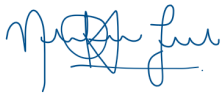
- (●) **The manuscript/published work is published or in press**

  Please provide the full reference of the research output:
  Marshall AC, Vigolo V, De Marchi M, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2025. Effect of protein polymorphisms on milk composition, coagulation properties, and protein profile in dairy sheep. International Dairy Journal. 160: 106102. doi: https://doi.org/10.1016/j.idairyj.2024.106102

- ( ) **The manuscript is currently under review for publication**

  Please provide the name of the journal:

- ( ) **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:		Main supervisor's signature:	

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Ana Carolina Marshall
Name and title of main supervisor:	Prof. Nicolas Lopez-Villalobos
In which chapter is the manuscript/published work?	Chapter 6

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Ana Carolina Marshall: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Nicolas Lopez-Villalobos: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Conceptualization. Simon M. Loveday: Writing – review & editing, Supervision. Mike Weeks: Writing – review & editing, Supervision. Warren McNabb: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Please select one of the following three options:

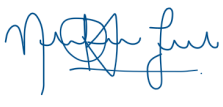
- (●) **The manuscript/published work is published or in press**

  Please provide the full reference of the research output:
  Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2024. Estimation of genetic parameters for production, composition and processability of milk from dairy sheep in a New Zealand flock. New Zealand Journal of Agricultural Research. Published online: 27 Jun 2024. https://doi.org/10.1080/00288233.2024.2368505

- ( ) **The manuscript is currently under review for publication**

  Please provide the name of the journal:

- ( ) **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:		Main supervisor's signature:	

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

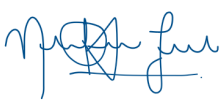
Student name:	Ana Carolina Marshall
Name and title of main supervisor:	Prof. Nicolas Lopez-Villalobos
In which chapter is the manuscript/published work?	Chapter 7

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Ana Carolina Marshall: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Vania Vigolo: Methodology. Massimo De Marchi: Methodology. Nicolas Lopez-Villalobos: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Conceptualization. Simon M. Loveday: Writing – review & editing, Supervision. Mike Weeks: Writing – review & editing, Supervision. Warren McNabb: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Please select one of the following three options:

○	**The manuscript/published work is published or in press** Please provide the full reference of the research output:
◉	**The manuscript is currently under review for publication** Please provide the name of the journal: Animal Biotechnology
○	**It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:		Main supervisor's signature:	

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Ana Carolina Marshall
Name and title of main supervisor:	Prof. Nicolas Lopez-Villalobos
In which chapter is the manuscript/published work?	Supplementary Information

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Ana Carolina Marshall: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Nicolas Lopez-Villalobos: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Conceptualization. Simon M. Loveday: Writing – review & editing, Supervision. Mike Weeks: Writing – review & editing, Supervision. Warren McNabb: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Please select one of the following three options:

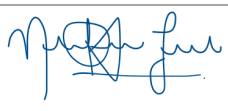
- (●) **The manuscript/published work is published or in press**

  Please provide the full reference of the research output:
  Marshall AC, Lopez-Villalobos N, Loveday SM, Weeks M, McNabb W. 2023. Udder and teat morphology traits associated with milk production and somatic cell score in dairy sheep from a New Zealand flock. New Zealand Journal of Agricultural Research Special issue: The New Zealand Society of Animal Production Annual Conference. https://doi.org/10.1080/00288233.2023.2248929

- ( ) **The manuscript is currently under review for publication**

  Please provide the name of the journal:

- ( ) **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:		Main supervisor's signature:	

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.