

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**The gross composition, fatty acid composition, and processability of  
milk from cows milked once and twice a day under New Zealand  
grazing conditions**

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

Doctor of Philosophy

in

Animal Science

Massey University, Palmerston North, New Zealand

**Inthujaa Sanjayanj**

**2023**



## Abstract

In New Zealand, milking once a day (OAD) is practised by some farmers, with the majority of farmers milking twice a day (TAD). Once-a-day milking provides several benefits such as reduced labour cost, improved labour efficiency, improved health and reproductive performance of cows, and additional employment opportunities for farmers. Once-a-day milking causes a reduction in milk yield and modifies milk composition by increasing the fat and protein percentages. The main aim of this thesis was to study the effect of OAD milking on the gross composition, fatty acids composition, and processability of milk. To achieve this, the milk from 232 cows from a OAD herd and 182 cows from a TAD herd were collected in a full season in early, mid-, and late lactation. The samples were analysed for milk composition and fatty acid composition using Fourier-transform mid-infrared (FT-MIR) spectroscopy. Calibration equations for individual and grouped fatty acids were developed using FT-MIR calibrator software. Milk coagulation properties (rennet coagulation time, curd firming rate, and curd firmness) were analysed using a Formagraph. In addition, a SNP (rs109421300) in the diacylglycerol acyltransferase 1 (DGAT1) region on bovine chromosome 14, which is associated with a higher fat percentage in milk, was selected to study its association with fatty acid composition. Solid fat content at 10°C (SFC<sub>10</sub>), a parameter that predicts butter hardness, was calculated using an equation developed from the proportions of fatty acids. The proportions of *de novo* synthesised fatty acids (C8:0-C14:0) were significantly higher, and the proportions of preformed, long-chain fatty acids (mainly C16:0 and C18:0) were lower in milk from cows milked OAD compared with cows milked TAD. Milk coagulation properties were superior for Jersey cows compared with Holstein-Friesian and Holstein-Friesian × Jersey cows milked OAD and TAD. The CC genotype of SNP rs109421300 was associated with higher fat yield, fat percentage, protein percentage, and SFC<sub>10</sub>. Milk from OAD cows had lower proportions of C16:0 suggesting it would produce more easily spreadable butter. Selecting cows with the CC genotype would be suitable for the New Zealand dairy industry with the current payment system, however, the CC genotype was less suitable for making more easily spreadable butter. Overall, there were some significant differences noted between milk from cows milked OAD and TAD in terms of fatty acid composition and processability.



*“No one will be able to stand against you all the days of your life.*

*As I was with Moses, so I will be with you; I will never leave you nor forsake you.”*

*- Joshua 1:5, NIV*

***Dedicated to Savinth***



## **Declaration**

This thesis contains no material that has been accepted for a degree or diploma by the University or any other institution. To the best of my knowledge, no material previously published or written by another person has been used, except where due acknowledgment has been made in the text. This thesis has been written with chapters formatted as papers for publication. Therefore, there is some repetition of chapter introductions, materials and methods, and references cited. Each chapter includes a full discussion, with the final general discussion chapter providing a discussion of the key findings of this thesis and their implications. Each chapter has been formatted for MDPI Journals and each has a complete list of references.



## Acknowledgements

First and foremost, I would like to praise and thank the almighty God for his blessings and mercy during my PhD study.

Words cannot express my gratitude to my supervisors, Prof Nicolas Lopez-Villalobos, Prof Hugh Blair, Dr Patrick Janssen, and Dr Steve Holroyd. Without your expertise, constructive feedback, support, guidance, and motivation, I could not have undertaken this journey. Nicolas, I am extremely grateful to you for accepting me as one of your students and teaching me genetics, mathematics, and statistics which strongly contributed to the success of this study. I am also thankful for your patience and support throughout this journey. Prof Blair, I am deeply indebted to you for providing me with motivation, guidance, and critical feedback and for teaching me genetics and statistics. Pat, I am sincerely thankful to you for sharing your enormous knowledge of food science and for training me in analysis. Steve, I am truly grateful for sharing your expertise in spectroscopy and for training me in analysis.

I would like to express my deepest appreciation to Dr Alastair MacGibbon for being a co-author of my research publications and for providing his prodigious knowledge to improve this research.

I am deeply grateful to the Accelerating Higher Education Expansion and Development Program (AHEAD), Sri Lanka for providing me with the scholarship to meet the expenses of studies and living, and to Massey University for finding the research part of this study.

I would like to offer my sincere thanks to Dr Jennifer Burke, Dr Rao Dukkupati, and Prof Dorian Garrick for teaching me dairy production, genetics, and breeding. I am also grateful to Prof David Thomas, Dr Thiagarajah Ramilan, and Dr Paramsothy Jeyakumar for their support in this PhD journey.

I would like to sincerely thank Fiona Sharland, Agricultural Research Technician, for helping me with the sampling. Fiona, your expertise and support in this study were crucial. I am also thankful to Massey University dairy farm staff, Jolanda Amooore, Lisa Whitfield, and Joshua Mitchell for helping me with fieldwork and data collection. Huge thanks to Felicity Jackson and the laboratory staff, Michelle Tamehana, Warwick Johnson, and Steve Glasgow for helping me with the laboratory analysis. Many thanks to

Sharon Wright, Ali Hagen, and Tara Penketh for your administrative support throughout my postgraduate study.

I take this opportunity to thank Eastern University, Sri Lanka for granting me study leave to pursue my PhD study and for their support in many ways.

Many thanks to my colleagues and friends, Shanika, Komahan, Dinesha, Priyanath, Dr Laura David, David Sathananthan, Dr Geretharan Thangavelautham, Dr Thivakary Geretharan, Dr Sarmini Maheswaran, Dr Chanjief Chandrakumar, Dr Martin Correa Luna, Dr Nipuna Perara, Dr Gayani Herath, Dr Pavithra Ariyaratne, Dr Sagara Kumara, Dr Bawatharani Raveendrakumaran, Nuwan, Sivajanani, Kamalam, Ana, Marit, Thi, Dr Mandefrot Meaza Zeleke, Dr Emmanuelle Haslin, Dr Natasha Jaques, Tony for accompanying and supporting me in this journey.

Most importantly, none of this could have happened without my family. I value and respect the support and love of my family. I would like to offer my special thanks to my husband Sanjay for all his understanding, motivation, sacrifices, support, and patience throughout this time. A huge thanks to my loving son Savinth for accepting my busy times and supporting me to achieve this breakthrough. I cannot forget the time you both spent on the farms and in the labs. I owe a debt of gratitude to my mother Ramani Yogarajah and my younger brother Sinthujan Yogarajah for their love and support. I would like to take this moment to remember my father Mr. (Late) Yogarajah Letchumanan and my elder brother Mr. (Late) Rajeevan Yogarajah whose memories always inspire me to pursue my studies.

## Contents

	Abstract.....	i
	Declaration.....	v
	Acknowledgment.....	vii
	Contents.....	ix
	List of tables.....	xi
	List of figures.....	xv
	List of abbreviations.....	xvii
Chapter 1	General introduction.....	1
Chapter 2	Literature review.....	9
	Foreword to Chapter 3.....	51
Chapter 3	Fatty acid composition of dairy milk: A case study comparing once- and twice-a-day milking of pasture-fed cows at different stages of lactation.....	53
	Foreword to Chapter 4.....	83
Chapter 4	Effect of breed on the fatty acid composition of milk from dairy cows milked once and twice a day in different stages of lactation.....	85
	Foreword to Chapter 5.....	107
Chapter 5	Association of single nucleotide polymorphism in the DGAT1 gene with the fatty acid composition of cows milked once and twice a day.....	109
	Foreword to Chapter 6.....	133
Chapter 6	A study of milk composition and coagulation properties of Holstein-Friesian, Jersey, and their cross milked once or twice a day.....	135
Chapter 7	General discussion.....	159



### List of tables

Table 2.1.	Percentages of different milk products exported as a fraction of total dairy exports from the New Zealand dairy industry in June 2020.....	11
Table 2.2.	Comparison of yield and composition between Holstein-Friesian (F), Holstein-Friesian $\times$ Jersey (F $\times$ J), and Jersey (J) cows in New Zealand.....	13
Table 2.3.	A summary of levels of production loss during once-a-day milking (OAD) compared with twice-a-day (TAD) milking in both short-term and full-lactation studies.....	16
Table 2.4.	Fatty acid composition of New Zealand milk fat.....	23
Table 3.1.	Dietary and chemical composition of feed offered at the No. 1 and No. 4 Dairy farms during the sampling periods (early, mid-, and late lactation) in the 2020–2021 production season.....	58
Table 3.2.	Descriptive statistics of daily yields of milk, fat, protein, and lactose, gross milk composition, and fatty acid composition of cows milked during the 2020–2021 production season.....	61
Table 3.3.	Least-square means and standard errors of daily yields of milk, fat, protein, and lactose, gross milk composition, and fatty acid composition of cows milked once a day (OAD) and twice a day (TAD) during the 2020–2021 production season.....	62
Table 3.4.	Least-square means and standard errors of daily yields of milk, fat, protein, and lactose, gross milk composition, and fatty acid composition of cows milked once a day (OAD) and twice a day (TAD) in early, mid-, and late lactation during the 2020–2021 production season.....	65
Table 4.1.	Means, standard deviations, and F-value for factors affecting production traits, milk composition, and fatty acid composition of cows milked once a day and twice a day during the 2020–2021 production season.....	92
Table 4.2.	Least-square means and standard errors for the production traits, milk composition, and fatty acid composition of milk from Holstein-Friesian (F), Holstein-Friesian $\times$ Jersey (F $\times$ J), and	

	Jersey (J) cows milked once a day (OAD) and twice a day (TAD) in the 2020–2021 production season.....	97
Table 5.1.	Feed allocation and chemical composition of diet provided at the No. 1 Dairy and No. 4 Dairy during the sampling period in the 2020–2021 production season .....	113
Table 5.2.	Descriptive statistics of production traits, gross milk composition, and fatty acid composition of cows milked once a day (OAD) and twice a day (TAD) during the 2020–2021 production season.....	116
Table 5.3.	Partial correlation coefficients between fat percentage and fatty acid composition of cows milked once a day (OAD) and twice a day (TAD).....	118
Table 5.4.	Least-square means and standard errors for the association between milk production traits, milk composition, and fatty acid composition and SNP rs109421300 genotypes for cows milked once a day (OAD) and twice a day (TAD).....	119
Table 6.1.	Dietary and chemical composition of feed offered at the No. 1 and No. 4 Dairy farms during the sampling periods (late lactation) in the 2020–2021 production season.....	139
Table 6.2.	Descriptive statistics of milk composition and milk coagulation properties of cows sampled during late lactation in the 2020–2021 production season.....	142
Table 6.3.	The F-values for factors affecting milk composition and milk coagulation properties of cows milked once a day and twice a day sampled in the morning and afternoon during the late lactation in the 2020–2021 production season.....	142
Table 6.4.	Least-square means and standard errors of milk composition and coagulation properties of milk from Holstein-Friesian (F), Holstein-Friesian × Jersey (F×J), and Jersey (J) cows milked once a day (OAD) and twice a day (TAD) sampled in the morning and afternoon during the late lactation in the 2020–2021 production season.....	144

Table 6.5. Partial correlation coefficients between coagulation properties and milk components of cows milked once a day during the late lactation in the 2020–2021 production season.....	145
Table 6.6. Partial correlation coefficients between coagulation properties and milk components of cows milked twice a day morning above the diagonal and afternoon below the diagonal during the late lactation in the 2020–2021 production season.....	146
Table 6.7. Partial correlation coefficients between coagulation properties and milk components of cows (regardless of milking frequency and milking time) during the late lactation in the 2020–2021 production season.....	146



## List of figures

Figure 2.1.	Practice of different milking intervals in New Zealand.....	14
Figure 2.2.	Glycerol phosphate pathway showing the role of diacylglycerol acyltransferase (DGAT) enzymes in fatty acid synthesis.....	25
Figure 3.1.	Daily yields of (a) milk, (b) fat, (c) protein, and (d) lactose in cows milked OAD (...) and TAD (—) during the 2020–2021 production season.....	63
Figure 3.2.	Percentage of (a) fat, (b) protein, and (c) lactose in cows milked OAD (...) and TAD (—) during the 2020–2021 production season.....	64
Figure 3.3.	Proportions of (a) saturated fatty acids, (b) unsaturated fatty acids, and (c) polyunsaturated fatty in cows milked OAD (...) and TAD (—) during the 2020–2021 production season .....	66
Figure 3.4.	Proportions of (a) short-chain fatty acids, (b) medium-chain fatty acids, (c) and long-chain fatty acids in cows milked OAD (...) and TAD (—) during the 2020–2021 production season .....	66
Figure 3.5.	Body condition score of cows milked OAD (...) and TAD (—) on a 10-point scale during the 2020–2021 production season.....	67
Figure 4.1.	Daily yield of (a) milk, (b) fat, (c) protein, and (d) lactose in milk from Holstein-Friesian (...), Holstein-Friesian × Jersey (...), and Jersey (...) cows milked OAD, and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season .....	93
Figure 4.2.	Percentages of (a) fat, (b) protein, and (c) lactose in milk from Holstein-Friesian (...), Holstein-Friesian × Jersey (...), and Jersey (...) cows milked OAD, and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season. ....	94
Figure 4.3.	Proportion of (a) C16:0 (b) C18:0 (c) C18:1 <i>cis</i> -9 in milk from Holstein-Friesian (...), Holstein-Friesian × Jersey (...), and Jersey (...) cows milked OAD, and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season. ....	95

Figure 4.4.	Proportions of (a) short-chain fatty acids (b) medium-chain fatty acids (c) long-chain fatty acids in milk from Holstein-Friesian (....), Holstein-Friesian × Jersey, (....) and Jersey (....) cows milked OAD, and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season.....	95
Figure 4.5.	Body condition score of Holstein-Friesian, Holstein-Friesian × Jersey, and Jersey cows milked OAD (....) and Holstein-Friesian and Holstein-Friesian × Jersey cows milked TAD (—) on a 10-point scale during the 2020–2021 production season.....	98
Figure 5.1.	The proportions of variance explained by the SNP rs109421300 for the production traits and composition traits in cows milked in the 2020–2021 production season .....	117
Figure 5.2.	Proportion of (a) C4:0, (b) C16:0, and (c) C18:1 <i>cis</i> -9 for genotypes CC (....), CT (....), and TT (....) in cows milked once a day, and proportions of (d) C4:0, (e) C16:0, and (f) C18:1 <i>cis</i> -9 for genotypes CC (—), CT (—), and TT (—) in cows milked twice a day during the 2020–2021 production season .....	121
Figure 5.3.	Solid fat content at 10°C (SFC <sub>10</sub> ) for genotypes CC (....), CT (....), and TT (....) in cows milked once a day (a), and for genotypes CC (—), CT (—), and TT (—) in cows milked twice a day (b) during the 2020–2021 production season .....	122
Figure 6.1.	The pattern of curd firming after rennet addition of milk from cows milked once a day (OAD) and twice a day (TAD) (morning and afternoon) in the late lactation in the 2020–2021 production season.....	147
Figure 7.1.	Proportions of (a) predicted solid fat content at 10°C of milk from cows milked once a day (OAD) (....) and twice a day (TAD) (—) and (b) predicted solid fat content at 10°C of milk from Holstein-Friesian (....), Holstein-Friesian × Jersey (....), and Jersey (....) cows milked OAD and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season.....	164

**List of abbreviations**

BCS = Body condition score

DGAT = Diacylglycerol acyltransferase

F = Holstein-Friesian

F×J = Holstein-Friesian × Jersey

J = Jersey

LCFA = Long-chain fatty acids

MCFA = Medium-chain fatty acids

MCP = Milk coagulation properties

MF = Milking frequency

OAD = Once a day

PUFA = Polyunsaturated fatty acids

RCT = Rennet coagulation time

SCFA = Short-chain fatty acids

SFA = Saturated fatty acids

SFC<sub>10</sub> = Solid fat content at 10°C

SNP = Single nucleotide polymorphism

TAD = Twice a day

UFA = Unsaturated fatty acids



## **Chapter 1**

### **General introduction**



The dairy industry has played a significant role in the New Zealand economy. The New Zealand dairy industry is driven by seasonal calving and a pasture-based farming system. Seasonal calving is well-synchronized with pasture growth to allow the cows to access the pasture as the main feed source throughout the season [1]. New Zealand dairy farmers traditionally have accepted twice-a-day (TAD) milking as a standard milking interval. Recently many farmers have found worthiness in practicing once-a-day (OAD) milking either a full season or part of a season.

Once-a-day milking is the practice of milking cows once in 24 hours [2]. The major benefit of OAD milking is the reduction of farm costs [3]. Other potential benefits resulting from OAD milking include reduction of labour inputs, better utilization of milking plants, allowing time for better pasture management and better heat detection, and creation of additional employment opportunities for farmers [4]. In addition, OAD milking also improves the reproductive performance of cows and increases submission rates and final pregnancy rates [3,5]. However, the main concern associated with OAD milking is the reduction in milk yield. The average yield losses were 22% in short-term experiments and 50% in full-season studies [6]. Milk solids yield produced in OAD milking was reduced by the range of 21-30% per hectare depending on the breeds [3,7]. Once-a-day milking also modifies the milk composition by increasing the fat and protein percentages and decreasing the lactose percentage [3,7,8]. Several studies report the effect of OAD milking on protein composition [9-11]. However, the effect of OAD milking on milk fat composition has not been investigated yet in New Zealand or overseas.

Knowledge of the fatty acid composition of milk is valuable for milk producers and milk processing companies to improve the quality of milk and dairy products. The fatty acid composition of milk determines the nutritional, sensory, and textural properties of dairy products, especially butter [12,13]. Recently, interest has been growing to determine the fatty acid composition of milk as it has been an increasingly economically important component of milk.

Dietary recommendations indicate that milk and dairy foods are an important part of a healthy balanced diet. Milk contains fat, protein, lactose, minerals, and vitamins [14]. In New Zealand, cow milk contains on average 4.75% fat [15] and fat consists of 98% triglycerides [16]. Triglycerides are made up of glycerol and fatty acids. Cow milk triglycerides contain around unique 400-500 fatty acids [17], among them, saturated fatty

acids from C4:0 to C18:0 and unsaturated fatty acids of C18 are major fatty acids. Many factors are associated with the variation in the composition and proportion of fatty acids in the milk. Their nature is mainly related to diet [18,19], stage of lactation [20,21], breed [22-24], and genetic variation [23-25]. It has been reported that the most significant difference in fatty acid composition is between Holstein-Friesian and Jersey cows. The New Zealand dairy industry's national herd consists of predominantly Holstein-Friesian, Holstein-Friesian × Jersey cows, and Jersey cows, and other breeds.

Few studies have reported a significant interaction between breed and milking frequency. Holstein-Friesian and Jersey cows milked OAD produce on average 30% and 21% lower milksolids (fat + protein) per cow compared with their counterparts who milked TAD [3,7]. The stage of lactation is another important factor that affects the composition of milk and consequently the processability of milk [26]. A few studies also indicated that the production and compositional changes due to OAD milking were dependent on the stage of lactation [27,28]. Therefore, it is important to study the effects of the interactions of breed and milking frequency, and the stage of lactation and milking frequency on fatty acid composition.

As New Zealand farmers show increasing interest in OAD milking it is important to conduct more studies on production traits and milk composition as they are factors in the milk payment system and influence the quality of the milk products. The general aim of this thesis was to investigate the fatty acid composition and processability of milk from cows milked OAD and TAD under New Zealand grazing conditions.

To achieve this general aim, this thesis addressed four specific objectives:

1. To compare the gross composition and fatty acid composition of milk from cows milked OAD and TAD at different stages of lactation.
2. To examine the milk composition and fatty acids composition of different breeds of cows milked OAD and TAD in different stages of lactation.
3. To investigate the effect of single nucleotide polymorphism (SNP) rs109421300, which is positively associated with fat content, on the fatty acid composition and processability of cows milked OAD and TAD.
4. To investigate the effect of breed on the composition and coagulation properties of milk from cows milked OAD and TAD morning and afternoon.

The results from this study will provide dairy farmers and milk processing companies with information about the fatty acid composition and how they affect the processability of milk. Moreover, the results would be helpful to make decisions on selecting suitable milking frequency and breed for making cheese and butter. It also has the potential to be considered by farmers who are willing to switch to OAD milking and select a breed proportion suitable for the OAD milking system. Furthermore, this research will provide a foundation for future research on the effect of the fatty acid composition of OAD milk on dairy product development.

## References

1. LIC. 2020. NZ dairy industry. <https://licnz.com/about/nz-dairy-industry/> Accessed on 8th February 2023.
2. DairyNZ. 2017. Once-a-day milking. <https://www.dairynz.co.nz/milking/milking-intervals/once-a-day-milking/> Accessed on 8th February 2023.
3. Clark, D.A.; Phyn, C.V.C.; Tong, M.J.; Collis, S.J.; Dalley, D.E. A systems comparison of once-versus twice daily milking of pastured dairy cows. *J. Dairy Sci.* **2006**, *89*, 1854–1862. [https://doi.org/10.3168/jds.S0022-0302\(06\)72254-8](https://doi.org/10.3168/jds.S0022-0302(06)72254-8)
4. Bewsell, D.; Clark, D.A.; Dalley, D.E. Understanding motivations to adopt once-a-day milking amongst New Zealand dairy farmers. *J. Agric. Educ. Ext.* **2008**, *14*, 69–80. <https://doi.org/10.1080/13892240701820611>
5. LIC. 2018. Once-a-day milking and reproduction. <https://www.lic.co.nz/support-and-advice/reproduction/once-day-milking-and-reproduction/> Accessed on 8th February 2023.
6. Stelwagen, K.; Phyn, C.V.V.; Davis, S.R.; Guinard-Flament, J.; Pomiès, D.; Roche, J.R.; Kay, J.K. Invited review: Reduced milking frequency. Milk production and management implications. *J. Dairy Sci.* **2013**, *96*, 1–13, <https://doi.org/10.3168/jds.2012-6074>
7. Tong, M.J.; Clark, D.A.; Cooper, C.V. Once-a-day milking: Possible and profitable? *Proc. N. Z. Grassl. Assoc.* **2002**, *64*, 33–37, <https://doi.org/10.33584/jnzg.2002.64.2470>
8. Lembeye, F.; López-Villalobos, N.; Burke, J.L.; Davis, S.R. Estimation of breed and heterosis effects for milk traits and somatic cell scores in cows milked once and twice daily in New Zealand. *Proc. N. Z. Soc. Anim. Prod.* **2015**, *75*, 60–63.

9. O'Brien, B.; Ryan, G.; Meaney, W.J.; McDonagh, D.; Kelly, A. Effect of frequency of milking on yield, composition, and processing quality of milk. *J. Dairy Res.* **2002**, *69*, 367–374, <https://doi.org/10.1017/S0022029902005605>
10. Pomiés, D.; Martin, B.; Chilliard, Y.; Pradel, P.; Rémond, B. Once-a-day milking of Holstein and Montbéliarde cows for 7 weeks in mid-lactation. *Animal* **2007**, *1*, 1497–1505, <https://doi.org/10.1017/S1751731107000778>
11. Martin, B.; Pomiés, D.; Pradel, P.; Verdier-Metz, I.; Rémond, B. Yield and sensory properties of cheese made with milk from Holstein or Montbéliarde cows milked twice or once daily. *J. Dairy Sci.* **2009**, *92*, 4730–4737. <https://doi.org/10.3168/jds.2008-1914>.
12. MacGibbon, A.K. Herd-to-herd variations in the properties of milkfat. *Proc. N. Z. Soc. Anim. Prod.* **1996**, *56*, 224–227.
13. Mackle, T.R.; Petch, S.F.; Bryant, A.M.; Auldist, M.J.; Henderson, H.V.; MacGibbon, A.K.H. Variation in the characteristics of milkfat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *N. Z. J. Agric. Res.* **1997**, *40*, 349–359, <https://doi.org/10.1080/00288233.1997.9513254>
14. Muehlhoff, E.; Bennett, A.; McMahon, D. *Milk and dairy products in human nutrition*, Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2013.
15. LIC and DairyNZ. 2021. New Zealand Dairy Statistics 2020-21. <https://www.dairynz.co.nz/media/5794941/nz-dairy-statistics-2020-21-web.pdf>  
Accessed on 21<sup>th</sup> December 2022.
16. Jensen, R.G. 2002. The composition of bovine milk lipids. *J. Dairy Sci.* **2002**, *85*, 295-350. [https://doi.org/10.3168/jds.S0022-0302\(02\)74079-4](https://doi.org/10.3168/jds.S0022-0302(02)74079-4)
17. Månsson, H.L. Fatty acids in bovine milk fat. *Food Nutr. Res.* **2008**, *52*, 1821. <https://doi.org/10.3402/fnr.v52i0.1821>
18. Palmquist, D.L.; Beaulieu, A.D.; Barbano, D.M. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* **1993**, *76*, 1753–1771, [https://doi.org/10.3168/jds.S0022-0302\(93\)77508-6](https://doi.org/10.3168/jds.S0022-0302(93)77508-6)
19. Dewhurst, R.J.; Shingfield, K.J.; Lee, M.R.; Scollan, N.D. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Feed Sci. Technol.* **2006**, *131*, 168–206. <https://doi.org/10.1016/j.anifeedsci.2006.04.016>

20. Stoop, W.M.; Bovenhuis, H.; Heck, J.M.L.; van Arendonk, J.A.M. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *J. Dairy Sci.* **2009**, *92*, 1469–1478, <https://doi.org/10.3168/jds.2008-1468>.
21. Kgwatalala, P.M.; Ibeagha-Awemu, E.M.; Mustafa, A.F.; Zhao, X. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. *Anim. Genet.* **2009**, *40*, 609–615, <https://doi.org/10.1111/j.1365-2052.2009.01887.x>
22. Auldist, M.J.; Johnston, K.A.; White, N.J.; Fitzsimons, W.P.; Boland, M.J. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *J. Dairy Sci.* **2004**, *71*, 51–57. <https://doi.org/10.1017/s0022029903006575>
23. Soyeurt, H.; Dardenne, P.; Gillon, A.; Croquet, C.; Vanderick, S.; Mayeres, P.; Bertozzi, C.; Gengler, N. Variation in fatty acid contents of milk and milk fat within and across breeds. *J. Dairy Sci.* **2006**, *89*, 4858–4865. [https://doi.org/10.3168/jds.S0022-0302\(06\)72534-6](https://doi.org/10.3168/jds.S0022-0302(06)72534-6)
24. Lopez-Villalobos, N.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Holroyd, S.E.; MacGibbon, A.K.; Snell, R.G. Estimation of genetic and crossbreeding parameters of fatty acid concentrations in milk fat predicted by mid-infrared spectroscopy in New Zealand dairy cattle. *J. Dairy Sci.* **2014**, *81*, 340–349. <https://doi.org/10.1017/S0022029914000272>
25. Stoop, W.M.; Van Arendonk, J.A.; Heck J.M.; Van Valenberg, H.J.; Bovenhuis, H. Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians. *J. Dairy Sci.* **2008**, *91*, 385-394. <https://doi.org/10.3168/jds.2007-0181>
26. Lucey, J. Cheesemaking from grass based seasonal milk and problems associated with late-lactation milk. *Int. J. Dairy Technol.* **1996**, *49*, 59–64. <https://doi.org/10.1111/j.1471-0307.1996.tb02491.x>
27. Stelwagen, K.; Knight, C.H. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. *J. Dairy Sci.* **1997**, *64*, 487–494, <https://doi.org/10.1017/s0022029997002458>
28. Davis, S.R.; Farr, V.C.; Stelwagen, K. Regulation of yield loss and milk composition during once-daily milking: A review. *Livest. Prod. Sci.* **1999**, *59*, 77–94, [https://doi.org/10.1016/S0301-6226\(98\)00204-8](https://doi.org/10.1016/S0301-6226(98)00204-8)



**Chapter 2**  
**Literature review**



## 2.1. New Zealand dairy industry

The New Zealand dairy industry is the world's eighth largest milk producer, producing approximately 21 billion litre/year, which is approximately 3% of global milk production [1]. The New Zealand dairy industry contains around 5,000,000 cows and plays an important role in the economy, accounting for around 3% of the gross domestic product [2]. The New Zealand dairy industry operates successfully with the highest milk volume and milk solids production reported in the 2020/2021 season compared with previous seasons: milk volume increased by 2.6% in litres and milk solids increased by 2.7% in kilograms [3]. Payments for the seasonal supply farmers are based on the "A+B-C" system where A is the value for fat yield, B is the value for protein yield and C is the penalty value for milk volume [3].

The New Zealand dairy industry produces more than 1500 dairy products and other product formulations such as milk powder, butter, cheese, infant formula, specialty cream for bakers, and products for sports and medical purposes [1]. New Zealand mainly exports its dairy products to China, Australia, the USA, Japan, and Malaysia [1]. The percentages of products exported by the New Zealand dairy industry are shown in Table 2.1.

**Table 2.1.** Percentages of different milk products exported as a fraction of total dairy exports from the New Zealand dairy industry in June 2020.

Product	Percentage
Whole milk powder	38
Butter and spread	17
Cheese and curd	11
Infant formula	9
Skim milk powder	8
Casein and caseinates	5
Milk and cream, not concentrated	5
whey	4
Other	3

Source: DCANZ [4]

New Zealand dairy farming is a seasonal pasture-based system running from June. Most of the herds in New Zealand start to calve in late winter/early spring (July to September). Following calving, milk production peaks and declines slowly through summer and autumn, and the cows are dried off when the milk yield starts to drop off to nearly 5-6 litres per day or when the next calving date is approaching [5]. In New Zealand, the period

between calving and drying off has been well-planned, corresponding to rapid and slow pasture growth [6]. Since it is a seasonal system, the calving interval should be maintained at 365 days and cows must become pregnant again in October and December [5].

New Zealand's climate, soil, and availability of water make it an excellent environment for growing grass [1]. The basic diet for dairy animals is pasture mainly ryegrass (*Lolium perenne*) generally combined with white clover species [5]. Different forages and crops such as chicory and plantain, legumes, brassica crops, fodder beet, and cereal crops are also fed to cows when pasture growth is inadequate during summer and cold winter months [5]. In addition, maize silage, pasture silage, palm kernel expeller, and concentrates are also used as supplementary feed. Moreover, adding nitrogen fertiliser provides additional feed by inducing pasture growth [7]. Although the use of supplementary feed has increased, typically 80% of the diet provided to cows is pasture [8]. Pasture growth, quality and nutritional content are associated with the season and cows' lactation stages. Energy requirements and milk production are also affected by the seasons and stages of lactation.

Although milk production is affected by feeding, it is also influenced by the genetic potential of the cows. To increase the rate of genetic gain and the ability to use bulls of high merit, artificial insemination is used; around 71% of the cows are artificially inseminated [3]. The dairy industry's national breeding objective is to select cows that are efficient converters of feed into profit [9]. Breeding worth is an index that has been developed to rank animals based on their expected ability to breed profitable and efficient progeny. The breeding index is calculated for nine important traits by combining breeding values and respective economic values. Genetic improvement plays an important role in economic improvement due to the emergence of molecular genetic techniques [10].

The national breed composition of the dairy industry in 2021 was 49.6% Holstein-Friesian  $\times$  Jersey (F $\times$ J), 32.5 % Holstein-Friesian (F), and 8.2% Jersey (J) cows [3]. These are the dairy breeds that dominate the New Zealand dairy industry. Holstein-Friesian cows are superior for milk volume compared with J cows and J cows are superior for the fat and protein contents compared with F cows. Holstein-Friesian  $\times$  Jersey cows are intermediate for milk volume and milk solids. Table 2.2 shows the yield and composition of F, F $\times$ J, and J cows in New Zealand.

**Table 2.2.** Comparison of yield and composition between Holstein-Friesian (F), Holstein-Friesian × Jersey (F×J), and Jersey (J) cows in New Zealand.

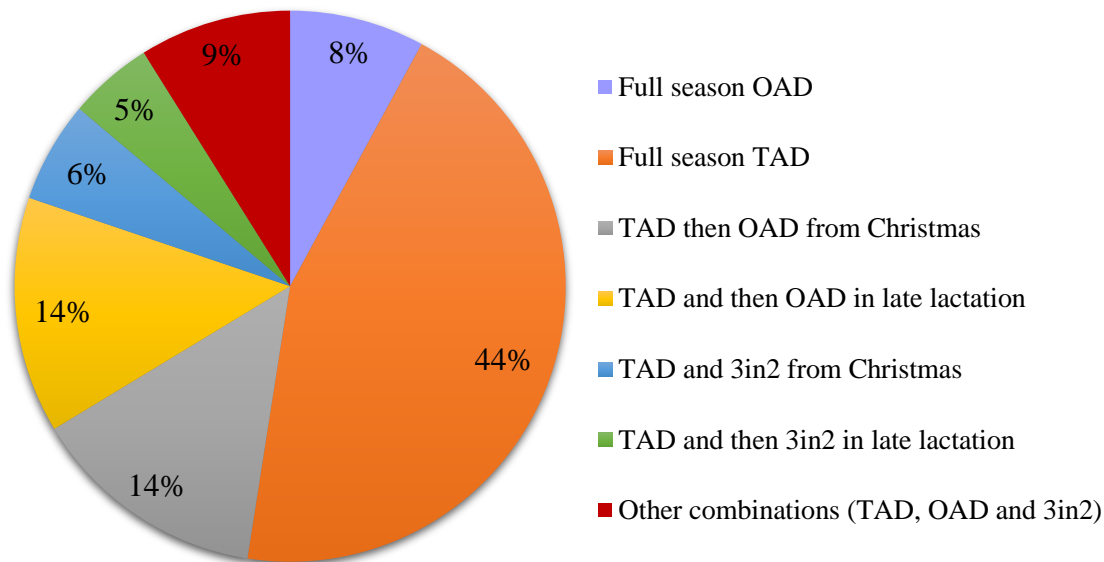
	Milk (litres)	Fat (kg)	Protein (kg)	Milk solids (kg)	Fat (%)	Protein (%)	Milk solids (%)
F	4764	211.8	180.2	392.0	4.5	3.8	8.3
J	3418	190.5	142.8	333.4	5.6	4.19	9.79
F×J	4325	212.3	172.0	384.3	4.95	3.99	8.94

Milk solids= fat + protein

Source: Adapted from LIC and DairyNZ [3].

Another important aspect of dairy farming is the milking frequency. In New Zealand traditionally twice-a-day milking (TAD) has been adopted by farmers. This means cows are usually milked in the morning and afternoon. Despite, TAD milking being traditionally accepted, only 45% of farms now practice TAD milking all year round [11]. Some farmers also have started to switch to once-a-day milking (OAD) for the full season or part of the season.

Once-a-day milking is the practice of milking cows once in the 24-hour. Once-a-day milking may be practiced for a full season or part of the season. In early lactation, OAD milking is practised to managing staff shortages, reducing stress on farmers during adverse weather [12], reducing metabolic stress during feed shortages [13], and managing the negative energy balance after calving during the period of feed shortage [14,15]. Once-a-day milking is practised in late lactation to manage the condition of the cows [13]. It is also common to practise OAD milking post-Christmas, i.e., slightly earlier than late lactation to extend the lactation with enough feed during the dry summer [12]. In New Zealand, 8% of the farms milk OAD for a full season, 28% of the farms milk OAD for part of the season, 45% milk TAD full season, and the remaining practise combination of TAD milking and three times in two days or combination of TAD milking, three times in two days and OAD milking (Figure 2.1, DairyNZ [11]). Once-a-day milking is becoming popular in New Zealand especially in some regions, for example, 31% of the farms in Northland milk OAD for a full season [12].



**Figure 2.1.** Practice of different milking intervals in New Zealand.

Source: Adapted from DairyNZ [11].

The growing popularity of OAD milking is due to its several benefits in terms of farm management, animals, and farmers. One of the major advantages of farm management is labour productivity. Labour cost has been identified as one of the highest expenses among farm expenses [16]. One of the major advantages of OAD milking is it reduces the labour inputs and increases the efficiency of labour resources [17] by allowing adequate time to spend on pasture management, heat detection, and animal welfare [18]. Minimizing time spent on milking also improves labour retention [17].

In terms of cows, OAD milking reduces heat stress, lameness (by reducing the walking time to and from the milking parlour), and standing time for milking [19,20]. Once-a-day milking also improves the negative energy balance in cows during the postpartum period and feed shortage [14,15]. Further, OAD milking also improves the body condition scores of cows [21,22]. Once-a-day milking also enhances the reproductive performance of cows by increasing the pregnancy rate [23], improving the 3-week artificial insemination submission rate, and reducing calving interval [22], which have positive effects on milk production. It is also reported that cows milked OAD showed lower dry matter intake compared with cows milked TAD [23,24]. In terms of farmers, OAD milking reduces stress on farmers regarding farming, provides time for additional employment options, and increases leisure time [25]. The main concern of OAD milking is the reduction of milk yield. The yield loss depends on the duration of OAD, breed, stage of lactation, and

parity [26]. The objective of this chapter was to review the effect of OAD milking on milk composition, fatty acid composition, and milk processability, which is related to the characteristics of butter and cheese.

## **2.2. Effect of once-a-day milking on milk yield**

Milk production loss is the most common and important aspect when cows are milked OAD. The extent of yield loss varies with the duration of OAD milking. As mentioned earlier, in practice, New Zealand farmers practise OAD milking for a short period as a part of lactation or full-time for the entire lactation. In short-term studies, the yield loss varies from 7-40%. Davis et al. [13] reviewed that the average milk yield loss in short-term studies has been 13%. Table 2.3 shows the yield loss in studies conducted on short-term and full lactation in New Zealand and overseas.

**Table 2.3.** A summary of levels of yield loss during once-a-day milking (OAD) compared with twice-a-day (TAD) milking in both short-term and full-lactation studies.

Reference	TAD milking yield (kg/day)	Yield loss (%)	Stage of lactation	Duration OAD milking	Breed
Claesson et al. [27]	-	50	All	Whole lactation	Swedish
Holmes et al. [24]	-	35	All	Whole lactation	NZ Holstein-Friesian and NZ Holstein-Friesian x Jersey cross and Jersey
	20	18	Mid	2 weeks	NZ Holstein-Friesian
	12-15	14-18		2 weeks	Jersey
Carruthers et al. [28]	15	13	Late	2 weeks	NZ Holstein-Friesian
	9-11	9-12		2 weeks	Jersey
	21	27	Early	12 weeks	NZ Holstein-Friesian
	13-15	10-21		12 weeks	Jersey
Knutson et al. [29]	14.50	27	Late	7 days	NZ Holstein-Friesian
Stelwagen et al. [30]	13	7	Late	2 weeks	Friesian
Auldism and Prosser [31]	21	19	Early	2 days	NZ Holstein-Friesian
Rémond et al. [21]	20.9	25	Early	3 weeks	French Holstein-Friesian
O'Brien et al. [32]	12.9	29	Late	10 weeks	Holstein-Friesian
Rémond et al. [33]	26.3	32	Early	3 weeks	French Holstein-Friesian
Rémond et al. [23]	24	30	All	Whole lactation	French Holstein-Friesian
Pomiès et al. [34]	20.2	22	Late	7 weeks	French Holstein-Friesian and French Montbéliard
Ferris et al. [35]	12.5	23	Late	79 days	Holstein-Friesian
McNamara et al. [36]	32	24.5	Early	4 weeks	Holstein-Friesian
Kay et al. [37]	22.65	21	Early	3 weeks	NZ Holstein-Friesian and NZ Holstein-Friesian x Jersey
Grala et al. [38]	14.9	20	Late	12 weeks	NZ Holstein-Friesian

In New Zealand and overseas there are some controlled full seasons studies conducted by Claesson et al. [27], Holmes et al. [24], Tong et al. [25], Rémond et al. [23], and Clark et al. [22]. These studies report a range of 22-50% and an average of 34% milk yield loss in OAD milking compared with TAD milking (reviewed by Stelwagen et al. [26]).

Some of the aforementioned studies also report a reduction in fat, protein, and lactose yields in cows milked OAD compared with cows milked TAD. In short-term studies, the extent of fat yield loss varied considerably and ranged from as little as 7% [30] to as high as 25% [36]. Similarly, protein yield loss in OAD milking ranged from 8% [30] to 25% [32]. Ferris et al. [35] reported a 19% relative loss of milk solid yield (fat yield + protein yield) in cows milked OAD. Once-a-day milking also causes a considerable reduction in lactose yield. Knutson et al. [29] and O'Brien et al. [31] reported a 29% and 31% reduction in lactose yield in cows milked OAD compared with cows milked TAD respectively. In the full lactation studies, Holmes et al. [24] reported a loss of 31% in fat yield, 24% in protein yield, and 37% in lactose yield, and Rémond et al. [23] reported losses of 25% in fat yield and 26% in protein yield in OAD milking relative to TAD milking. The variation in the results of these short-term and full lactation studies could be due to the differences in the management system, feed, breed, and stages of lactation.

Stelwagen et al. [29] reported yield loss in cows milked OAD would be due to the presence of a local intramammary inhibitor of milk secretion and rupture of tight junctions, a small extracellular membrane that prevents the leakage of milk into adjacent cells. Wilde et al. [39] reported that the inhibitor is called FIL (Feedback Inhibitor of Lactation), secreted by the mammary gland and released into milk, which reduces the rate of milk secretion when its concentration is higher. Carruthers et al. [27] reported that the reduction in yield due to OAD could be due to the loss of secretory cells and the loss of udder capacity. The reason for this may be the upregulation of the apoptotic gene [40,41]. Overall, these factors cause production loss in cows milked OAD. These physiological changes that occur in cows milked OAD could also modify the milk composition.

### **2.3. Effect of once-a-day milking on milk composition**

Once-a-day milking alters the composition of milk. Davis et al. [13] reviewed that OAD milking increases the fat and protein percentages and decreases the lactose percentage of milk. Similarly, in short-term studies, Stelwagen et al. [29]; Rémond et al. [21]; O'Brien et al. [31]; Ferris et al. [34]; Grala et al. [37] reported higher protein and fat percentages,

and lower lactose percentage in different stages of lactation and in different breeds. In contrast, in short-term studies, Kay et al. [36] did not report a difference in fat percentage in early lactation, Pomiès et al. [33] did not report variation in lactose percentage in late lactation, and Auldist and Prosser [30] reported no change in protein percentage in early lactation milk from cows milked OAD compared with cows milked TAD. Rémond et al. [32] reported no significant changes in fat, protein, and lactose percentages between OAD and TAD cows in early lactation. In full lactation studies, Claesson et al. [26] reported higher protein percentage, and Holmes et al. [24] reported lower lactose percentage in milk from cows milked OAD compared with cows milked TAD. These studies suggest that OAD milking modifies the milk composition however, it depends on the breed and stages of lactation.

Few studies also reported significant changes in the composition of protein in milk from cows milked OAD. O'Brien et al. [31] and Pomiès et al. [33] reported that the increase in protein percentage in cows milked OAD compared with cows milked TAD was due to the increase in whey protein content. O'Brien et al. [31] reported lower casein content in cows milked OAD compared with cows milked TAD. In contrast, Pomiès et al. [33] reported higher casein content in cows milked OAD, but the effect of OAD milking was higher on the whey content. Among the whey proteins,  $\beta$ -lactoglobulin content was higher, and  $\alpha$ -lactalbumin remained unchanged with OAD milking (reviewed by Davis et al. [13]). OAD milking also increased the serum albumin content [29], proteolytic enzymes [28,42], IgG content [30], and plasminogen content [28,31,33] in milk.

The changes in milk composition of cows milked OAD could be due to the change in permeability of tight junction between mammary epithelial cells and adjacent cells [13]. The reason for higher fat content is still not well-known. However, the concentration effect due to leakiness could be a reason [38]. Higher fat content may be related to the changes in the regulatory mechanisms for fat synthesis in terms of the aqueous phase of milk [13]. O'Brien et al. [31] reported that the increase in protein content may be due to the improved energy balance of cows milked OAD. Overall OAD milking modifies the gross composition and protein composition of milk.

## 2.4. Effect of once-a-day milking on processability

The modification of milk composition due to OAD milking may affect the processing properties of milk [24], however, there are only limited studies examining the effect of OAD milking on the processability of milk into cheese, butter, and other dairy products.

### 2.4.1. Cheese and milk coagulation properties

Milk coagulation and syneresis are important aspects of cheese making. Better coagulation of milk depends on good rennet activity, higher curd firming ability, and higher curd firmness. One method of measuring milk coagulation properties (MCPs) is using a Formagraph. A Formagraph measures rennet coagulation time (RCT, mm), curd firming time ( $A_{20}$ , mm) which is measured as the time taken to form 20 mm depth curd, and curd firmness 30 minutes after rennet addition ( $A_{30}$ , mm).

The mechanism of rennet coagulation of milk can be divided into three stages [43]. The primary stage is enzymatic hydrolysis where the rennet enzyme cuts off  $\kappa$ -casein to permit the aggregation of casein micelles. In the second stage, further hydrolysis of  $\kappa$ -casein takes place which changes the properties of casein micelles. Cleavage of  $\kappa$ -casein reduces the surface charge of the casein micelles and facilitates aggregation [44]. Freshly cut  $\kappa$ -casein is known as para-kappa-casein and binds with calcium and phosphate. These minerals form a bridge that links the casein micelles to form the gel. In the tertiary stage, the syneresis of the gel is started by the removal of water and a three-dimensional network is formed [45].

Milk coagulation properties are considered basic requirements for cheese making [46]. Milk that starts to coagulate soon after the addition of a clotting enzyme would form firmer curd with higher cheese yield [47]. Ikonen et al. [48] reported that Finnish Friesian cows producing good MCPs produced more Emmental Cheese with higher dry matter content and lower loss of fat and casein in whey compared with poorly coagulated milk. Malacarne et al. [49] reported a lower curd firming time ( $K_{20}$ ) in Italian Brown Swiss cows resulted in better rheological properties of Parmigiano-Reggiano cheese compared with the cheese made from Italian Friesian milk with higher  $K_{20}$ . Cecchinato and Bittante [50] reported that milk from Brown Swiss cows had higher curd firmness 45 minutes after rennet addition ( $A_{45}$ ), with a strong positive relationship between cheese yield and its components, milk solid yield, and water yield in cheese. These findings suggest that the cows which have better MCPs can produce cheese with good quality and yield. Milk

coagulation properties can be affected by breed, stage of lactation, diet, and milking frequency.

Variation in milk composition between breeds is well-known. Similarly, variations in MCPs and cheese characteristics between breeds were studied extensively. Auldism et al. [51] reported that the curd firming time of milk from J cows was lower than F cows, and it was most likely caused by the higher casein content of J milk when analysed as individual and bulk samples standardised for protein: fat ratio. Similar results were reported by Jensen et al. [52] in Danish Jersey and F cows.

Stocco et al. [53] reported that J cows showed better MCPs compared with F, Brown Swiss, Simmental, Rendena, and Alpine Gray cows. In an experiment comparing MCPs of mid-lactation F, Brown Swiss, Simmental, Rendena, and Alpine Gray cows, De Marchi et al. [54] reported F cows had the highest number of samples with poor MCPs and Rendena cows had the lowest, with better MCPs among other breeds. Martin et al. [55] reported Montbéliarde cows had shorter RCT and  $K_{20}$  compared with Holstein cows in a 7-week trial. Similarly, Montbéliarde cows produced more cheese from 100 kg of milk when compared with Holstein cows. The authors also reported the superiority of Montbéliarde cows over Holstein cows for MCPs was due to their higher casein and fat content in milk. De Marchi et al. [56] reported Brown Swiss cows showed better RCT and curd firmness compared with F cows in 12 cheese-making trials experimenting with 3 different kinds of cheese. Malacarne et al. [49] and Franceschi et al. [57] reported curd firming rate, curd firmness, cheese yield, and cheese-making parameters of Parmigiano Reggiano cheese made from Italian Brown cows were higher than Italian Friesian cows.

Several of the above-mentioned studies reported that good MCPs were associated with higher contents of protein, casein, and fat in milk. However, Auldism et al. [51] and De Marchi et al. [54] reported that good MCPs are not only due to higher protein or casein contents of milk but also other aspects which could be casein fractions [58, 59],  $\kappa$ -casein genetic variation [59,60],  $\beta$ -lactoglobulin [61], pH [62,63], titrable acidity [46,64], and calcium content [65,66]. Therefore, good MCPs depend on a combination of several factors.

Milk yield and milk composition vary with the stages of lactation [67-69]. Similarly, stages of lactation affect the MCPs and cheese characteristics. Auldism et al. [70] reported that cheese produced from late lactation milk was inferior in quality with lower fat and

protein recovery and poor flavour scores, compared with cheese made from early lactation milk, in a trial with milk collected from 14 commercial farms. Gulati et al. [69] reported that the low-moisture part-skim mozzarella cheese produced from the milk from F and F×J cows in late lactation had lower content of fat and protein compared with the cheese made from mid-lactation. Albenzio et al. [71] reported lower curd firmness in late lactation than in mid-lactation in ewes. Coulon et al. [72] reported the curd firming time decreased with progressing lactation, but the late lactation milk reached maximum firmness faster but that quickly decreased due to rapid syneresis.

Fresh cheese yield from late lactation was higher per 100 kg of milk compared with the cheese yield from early lactation milk [70,72]. The reason for higher cheese yield and poor fat and protein recovery could be due to the higher fat and protein content in late lactation milk [70,72]. Ostensen et al. [73] reported the changes in MCPs were associated with the underlying mechanism of producing milk protein, energy balance, feed intake, and proteolysis. The results of the above studies reveal that late lactation milk would be less suitable for cheese-making compared with early and mid-lactation. However, the results also depend on breed, feed, and type of cheese produced.

Cow diet is another important parameter that affects milk composition [74] and dairy products, mainly cheese [75,76]. Feeding different diets affects the texture, yield, and other characteristics of cheese. Carpino et al. [77] reported that pasture-fed cows produced cheese with more odour-active compounds compared with total mixed ration-fed cows. O'Callaghan et al. [78] reported that the cows fed with a total mixed ration produced harder cheddar cheese compared with cows fed with perennial ryegrass pasture only and perennial ryegrass and white clover diet in an experiment with Friesian cows. The reason for this was the higher proportion of C16:0 fatty acid and lower proportion of oleic acid in total mixed ration-fed cow milk. Gulati et al. [69] reported the feeding system had no or little effect on cheese composition and fat or protein recovery in whey, in part-skim Mozzarella cheese produced from cows grazing on perennial ryegrass and perennial ryegrass/white clover mixture compared with total mixed ration. These studies reveal that pasture-fed cheese is good in terms of quality and human health due to its texture and beneficial fatty acid composition. Apart from these common factors, milking frequency could also affect MCPs and cheese-making characteristics.

The modification of milk composition due to OAD milking may affect the processing properties of milk [24], however, there are only limited studies examining the effect of OAD milking on the processability of milk into cheese, butter, and other dairy products. Pomiès et al. [33] found that manufacturing good quality cheese from OAD milking was challenging due to prolonged rennet coagulation time and curd firmness caused by the higher protein percentage. However, O'Brien et al. [31] found variation only in curd firming time in F cows milked OAD for 10 weeks compared with cows milked TAD. Martin et al. [55] reported OAD milking did not cause any significant changes in the sensory characteristics of cheese. The relationship of milk fatty acid with coagulation properties was only reported in cows milked TAD by Auldist et al. [51] in F and J cows in a 10-day trial. Proportions of C8:0, C10:0, and C12:0 were positively correlated with the curd firming time and positively with the curd firmness. This suggests there are possibilities that milk composition and fatty acid composition of cows milked OAD could have associations with milk coagulation parameters. The reason for modifications in the MCPs could be partly the changes in the milk composition of OAD milk. Pomiès, et al. [33] studied the characteristics of OAD milk for 7 weeks in mid-lactation and reported that the total protein percentage and concentrations of whey, casein, calcium, and phosphorus were higher in OAD cows than in TAD cows. Similar results were observed by Lacy-Hulbert et al. [79] in a 26-day late lactation study. Casein micelles in milk are large and cannot move through the tight junction [80], therefore, they remain in the mammary gland and become concentrated after the movement of lactose and water in cows milked OAD [79].

Cheese-making depends on factors discussed here and some other factors such as somatic cell count, processing conditions, storage of milk, standardisation of milk, type of starter culture/ coagulant used, heat treatment of milk, and homogenisations of milk [81].

#### **2.4.2. Butter and fatty acids**

Milk fat is one of the complex components due to the presence of many fatty acids with different structures. Milk fat is made of approximately 98% of triglycerides [82]. Triglycerides are composed of glycerol and fatty acids. Fatty acids are made up of a carboxyl group and a hydrocarbon chain. Researchers have identified approximately 400 fatty acids in milk. Most of these fatty acids are present in less than 0.01% [83]. The fatty acid composition of New Zealand milk is shown in Table 2.4.

**Table 2.4.** Fatty acid composition of New Zealand milk fat.

	Fatty acid	% , w/w
C4:0	Butyric acid	3.0
C6:0	Caproic acid	1.9
C8:0	Caprylic acid	1.2
C10:0	Capric acid	2.8
C12:0	Lauric acid	4.5
C14:0	Myristic acid	12.1
C14:1	Myristoleic acid	0.9
C15:0	Pentadecylic acid	1.1
C16:0	Palmitic acid	30.4
C16:1	Palmitoleic acid	1.3
C18:0	Stearic acid	10.6
C18:1 <i>cis</i>	Oleic acid	18.8
C18:1 <i>trans</i>	Vaccenic acid	4.2
C18:2	Linoleic acid	0.8
C18:2 conj	conjugated linoleic acid	1.1
C18:3	Alpha linoleic acid	0.6
	Minor fatty acids	4.6

Source: MacGibbon [83]

Fatty acids have both a systematic and a trivial name and are mostly expressed using a shorthand formula with carbon numbers and the position of double bonds. For example, fatty acid C16:0 (shorthand formula) has 16 carbon atoms with no double bonds whereas fatty acid C18:1 *cis*-9 has 18 carbon atoms with a double bond in the 9<sup>th</sup> carbon. The fatty acids also have common names for instance C16:0 is called palmitic acid and C18:1 *cis*-9 is called oleic acid. Other than triglycerides milk fat consists of small proportions of diacylglycerol, cholesterol, phospholipids, and free fatty acids [84].

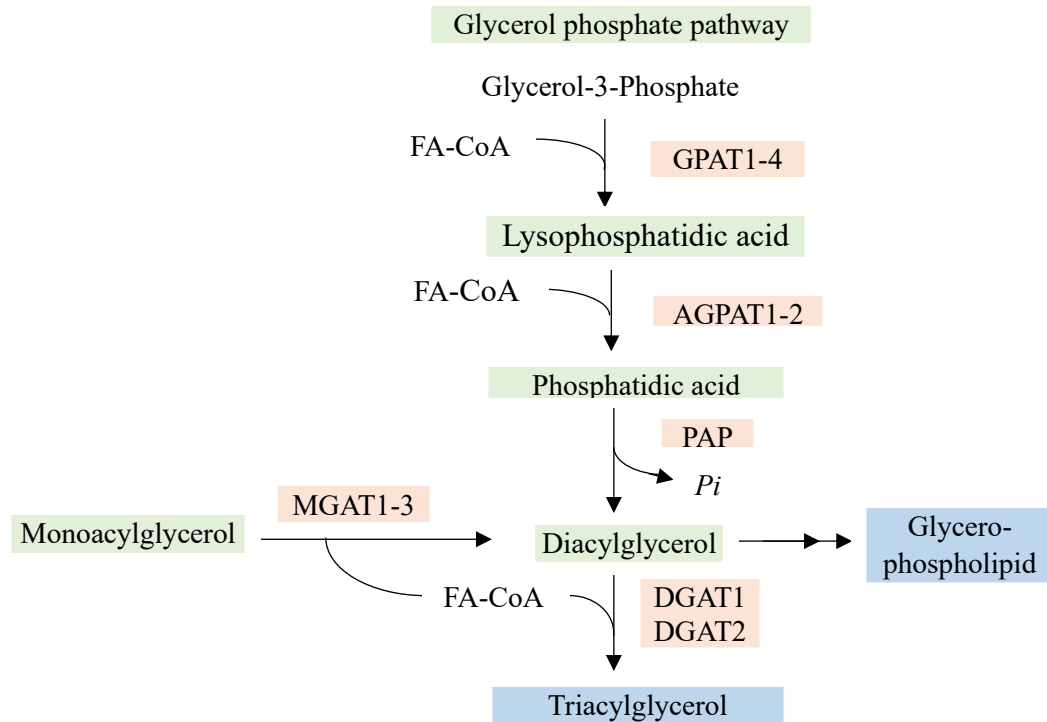
Fatty acids are classified into different classes based on saturation and unsaturation, length of the chain, odd and even number of the carbon chain, and odd and branched pattern of the carbon chain. Fatty acids have been categorized as saturated fatty acids (SFA) and unsaturated fatty acids (UFA) based on the presence or absence of double bonds. Saturated fatty acids do not contain any double bonds whereas UFA contains double bonds in their chains. Unsaturated fatty acids are further classified into monounsaturated fatty acids (MUFA) which contain single double bonds and polyunsaturated fatty acids (PUFA) which contain two or more double bonds. The percentage of SFA, MUFA, and PUFA present in cow milk are typically 70%, 25%, and

5%, respectively [85,86]. Saturated fatty acids are stable, chemically less reactive, and have a long life in the body [87].

Based on the length of the carbon chain fatty acids are classified into short-chain fatty acids (SCFA) (4-8 carbon atoms), medium-chain fatty acids (MCFA) (10-14 carbon atoms), and long-chain fatty acids (LCFA) (also known as preformed fatty acids, 16 and more carbon atoms). Fatty acids containing an even number of carbon atoms are named even-chain fatty acids and uneven numbers are named odd-chain fatty acids. The odd and branched-chain fatty acids are synthesized by specific bacteria in the rumen and these fatty acids are only up to 4% of total fatty acids. The UFA consists of double bonds in two configurations. In the *cis* configuration, the hydrogen atoms are attached to the double bonds on the same side, and in the *trans* configuration, the hydrogen atoms are on opposite sides. *Trans* fatty acids are only 2 to 6% of the total fatty acids present in ruminant reserves and milk fat [88]. *Trans* fatty acids are produced in the ruminant stomach by the hydrogenation of UFA during microbial fermentation [89].

Fatty acids originate from two sources: *de novo* synthesis in the mammary epithelial cells and direct uptake from diet and by a combination of both [90]. Short and medium-chain fatty acids (C4:0 to C14:0) and about half of the proportion of C16:0 are biosynthesized in the mammary epithelial cells. The remaining proportions of C16:0 and LCFA (C18:0–C22:0) are produced by the absorption from diet or the metabolization of adipose fat tissue.

*De novo* synthesis of fatty acids mainly uses acetate and butyric acid which are produced by microbial fermentation of cellulose and related components as precursors. Butyric acid is converted to  $\beta$ -hydroxybutyrate when absorbed through rumen epithelium [91]. Acetate is activated to acetyl-CoA and converted to malonyl-CoA by the process of carboxylation. The stepwise process of elongation takes place to produce a series of SCFA and MCFA [83]. Unsaturated fatty acids are mainly formed by adding double bonds into the SFA by the enzyme delta-9-desaturase or stearoyl-coenzyme A desaturase [92].



**Figure 2.2** Glycerol phosphate pathway showing the role of diacylglycerol acyltransferase (DGAT) enzymes in fatty acid synthesis.

FA-CoA - fatty acyl CoA; GPAT- glycerol-3-phosphate acyltransferase; AGPAT- acylglycerolphosphate acyltransferase; PAP - phosphatidic acid phosphatase; MGAT- monoacylglycerol acyltransferases.

Substrates and intermediates are shaded green, major products are shaded blue, and enzymes are shaded in orange.

Adapted from Yen et al. [93] and Gimeno and Cao, [94].

Triglycerides are major components of milk fat. They are formed by binding one molecule of glycerol with three molecules of fatty acids. Important enzymes that catalyse the final step of triglyceride synthesis are diacylglycerol acyltransferase (DGAT) enzymes [93,95]. The final step of converting diacylglycerol into triacylglycerol is catalysed by DGAT1 and DGAT2 enzymes [95,96] (Figure 2.2). Two genes encoding DGAT1 and DGAT2 enzymes are DGAT1 and DGAT2 [96].

Fatty acids composition is considered important in the processability of milk mainly in butter manufacturing. Approximately 80% of the variation in the textural properties of butter can be explained by the variation in fatty acid composition [97]. Milk containing higher proportions of UFA would lead to softer and more easily spreadable butter [98,99]. Bobe et al. [100] reported butter produced from milk from a herd containing mid-lactation multi-breed (Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, and Milking Shorthorn)

cows was more spreadable and softer due to higher proportions of UFA. Couvreur et al. [101] reported that increasing the amount of grass in the diet of mid-lactation Holstein cows resulted in softer butter and low solid fat content due to a lower ratio of C16:0 to C18:1. These studies suggest that the composition and proportions of fatty acids affect the spreadability of butter.

The spreadability or hardness of the butter is affected by the solid fat content of milk. Solid fat content is the ratio between solid fat and the total fat (sum of solid and liquid fat) in milk. Solid fat content measured at 10°C (SFC<sub>10</sub>) has a positive association with the spreadability of butter [102,103]. Milk fatty acid composition affects the SFC<sub>10</sub> of milk. MacGibbon [103] and Mackle et al. [104] reported a significant positive correlation between the proportion of C16:0 and SFC<sub>10</sub> and a significant negative correlation between C18:1 and SFC<sub>10</sub>. The effect of the fatty acid composition of cows milked OAD on butter characteristics has not been extensively studied. Butter composition, carotene and colour measurements, solid fat content, melting point, and water activity are some of the important physicochemical and rheological properties of butter [105]. Variations in fatty acid composition could affect the properties of butter. The fatty acid composition of milk is affected by several factors.

Most notable differences have been reported between breeds for fatty acid composition in milk. In an experiment with milk samples collected from 30 farms containing F and J cows for 2 days in mid-lactation, MacGibbon [103] reported that J cows had higher proportions of MCFA (C8:0 to C12:0), C14:0 and C18:0 and lower proportions of C18:1 and C18:2 *cis* fatty acids compared with F cows. Similar results were reported by Beaulieu and Palmquist [106] with J cows producing higher SCFA in a 2-week late lactation trial and by Lopez-Villalobos et al. [107] with F, F×J and J cows using more than 300 full-season lactation records of F×J cows. The aforementioned studies and other studies by Soyeurt et al. [108]; Croissant et al. [109]; Palladino et al. [110] reported that J cows produced higher proportions of SFA, and lower proportions of UFA compared with F cows. One of the reasons for the breed differences in fatty acids could be the activity of the delta-9-desaturase enzyme between breeds [111], which is the central enzyme in the production of UFA in milk. The activity of this enzyme is lower in J cows compared with F cows [112]. Holstein-Friesian × Jersey cows produced intermediate values for most of the fatty acids including the proportions of SFA and UFA [107,110].

Moreover, breed differences for fatty acids were reported in other breeds. Soyeurt et al. [108] reported dual purpose Belgian Blue cows produced lower proportions of SFA and optimum proportions of C18 UFA with higher activity of delta-9-desaturase enzyme compared with F, J, Montbeliarde, Normande, and non-Holstein Meuse-Rhine-Yssel type Red and White breeds. Moioli et al. [112] reported J cows produced higher proportions of SCFA and MCFA and lower proportions of C18 UFA compared with Piedmontese and Valdostana cows. Similar results were reported for J cows by Maurice-Van Eijndhoven et al. [113] when compared with F, Meuse-Rhine-Yssel, Dutch Friesian, Groningen White Headed, and J cows. Wales et al. [114] reported that compared with North American-origin F cows, New Zealand-origin F cows produced higher proportions of C6:0-C16:0 and lower proportions of C18 UFA due to lower activity of  $\Delta^9$ -desaturase enzyme. The reason for variations between breeds may be due to significant genetic variations in the proportions of some fatty acids and genes coding the enzymes catalysing fatty acids synthesis [111].

The other factor affecting milk fatty acid composition is the stages of lactation and which were extensively studied. Lactation has divided into three stages: early (<90 days in milk), mid (90-180 days in milk), and late (>180 days in milk). Several studies report the effect of the stage of lactation on milk fatty acids is prominent in the early weeks of early lactation [67,115]. In an experiment with Canadian Holstein cows in early, mid-, and late lactation, Kgwatalala et al. [116] reported lower proportions of C10:0-C16:0 and SFA, and higher proportions of C18:1 *cis*-9, MUFA, and PUFA in early lactation compared with either mid- or late lactation. In 16-week and 12-week studies Kay et al. [117] and Gross et al. [115] respectively, reported the proportions of *de novo* synthesised fatty acids were lower, and the proportions of LCFA (preformed fatty acids) were higher in week one of the post-partum, and then the proportions were opposite. Similar results were reported by Auld et al. [67] in New Zealand F cows, Garnsworthy et al. [118] in Holstein cows, Bilal et al. [119] in Canadian Holstein cows, Schwendel et al. [120] in New Zealand multi-breed herd containing mainly F and J cows, and Churakov et al. [121] in Swedish red cows. The aforementioned studies also reported increasing proportions of *de novo* fatty acids and decreasing proportions of LCFA in mid-lactation. Proportions of UFA were lower in mid-lactation compared with early and late lactation in Dutch Holstein-Friesian cows [122], New Zealand F cows [123], and New Zealand multi-breed herd containing F, F×J and J cows [124]. Significant changes in the fatty acid composition

of milk in early lactation particularly immediately after calving are due to negative energy balance [115,116].

The increase in the energy requirement for foetal development and milk production may lead to a negative energy balance. The energy balance of the cows and utilization of energy reserves are reflected in the fatty acid composition of milk [125]. On average, preformed fatty acids contribute larger proportions in the total fatty acids in the early lactation milk and, as the lactation progresses, *de novo* fatty acid starts to show higher proportions in milk [74]. Due to negative energy balance cows use the energy stored in the adipose tissue and this leads to higher proportions of LCFA, mainly C18 and C18:1 *cis*-9, in the milk [115-117]. Higher proportions of LCFA mobilised from adipose tissue inhibit the synthesis of *de novo* fatty acids in the mammary gland [74,126].

In general, the fatty acid composition of early lactation milk is considered more desirable for making easily spreadable butter as it contains higher proportions of UFA, mainly C18:1 *cis*-9 [119]. However, Schwendel et al. [120] reported that the proportion of C18:1 *cis*-9 was not affected by the stage of lactation as the cows were adequately fed and in better energy balance. This suggests diet contributes to considerable variation in the fatty acid composition of milk.

Manipulation of animal diet affects the fatty acid composition of milk. Cows fed with pasture tend to yield milk with beneficial fatty acids in terms of milk processing and human health. It is widely accepted incorporating pasture in cows' diets increases the beneficial fatty acid in milk compared with including a total mixed ration. In an experiment comparing grazing pasture and providing a total mixed ration, Santa et al. [127] reported that the cows grazed on pasture produced higher proportions of alpha-linolenic acid, conjugated linoleic acid, MUFA, and PUFA compared with feeding total mixed ration. The authors also reported that the proportions of fatty acids C12:0, C14:0, C16:0, SFA, and n-6 fatty acids were higher in total mixed ration compared with pasture. Similar results were reported by White et al. [128], O'Callaghan et al. [129] and Barca et al. [130]. Pasture contains a higher proportion of PUFA. Intake of alpha-linolenic acid increases its proportion in milk, and the products released from the biohydrogenation of alpha-linolenic acid in the rumen lead to inhibit the synthesis of SFA mainly C12:0, C14:0, and C16:0 [127]. This makes the milk with higher proportions of UFA.

In New Zealand, chicory is fed to cows in addition to pasture. Mangwe et al. [131] and Muir et al. [132] reported feeding chicory (*Cichorium intybus*) resulted in a higher proportion of linoleic acid, alpha-linolenic acid, and PUFA in milk compared with ryegrass. Similarly, preserved forages also affect the fatty acid composition of milk. In experiments comparing pasture silage and maize silage, Kliem et al. [133] and Hernandez-Ortega et al. [134] reported that the milk from cows fed with pasture silage contained a higher proportion of alpha-linolenic acid and lower proportions of n-6 fatty acids compared with maize silage. Reviews by Kalac et al. [135] and Hanus et al. [136] reported milk from cows fed with legume silage contained higher proportions of PUFA compared with grass silage. These studies expose that legume silage could result in beneficial fatty acids compared with maize silage, with pasture silage being intermediate. Similarly, adding dietary fat into animals' diets affects the fatty acid composition of milk. Mierlita et al. [137] reported supplementing milled rape seed with a high- or low-concentrate diet increased the proportions of MUFA and C18:1 *cis*-9, vaccenic acid and alpha-linolenic acid, and decreased the proportions of SFA compared with non-supplemented diet in J cows. Similar results were reported by Kleim et al. [138] and Mitsiopoulou et al. [139] that supplementing extruded linseed, calcium salts of palm, linseed oil, and milled rape seed in the diet of F cows and whole sesame seeds in goats, respectively. The literature suggests that a diet consisting of pasture, legumes, and seeds would give milk with higher proportions of UFA including alpha-linolenic acid and conjugated linoleic acid, and a lower proportion of SFA. Overall, these studies show cows fed with a pasture diet would have beneficial fatty acids in milk, by having a higher proportion of PUFA. This would provide benefits in butter making by reducing the hardness of the butter.

The diet not only influences the fatty acid composition of milk but also the characteristics of butter. Couvreur et al. [101] reported increasing the proportion of fresh grass in the corn silage diet of Holstein cows reduced the C16:0/C18:1 ratio of milk and reduced the hardness of butter. Increasing the proportion of fresh grass also reduced the rancid flavour of butter. Similarly, O'Callaghan et al. [129] reported Holstein cows fed ryegrass pasture and ryegrass/white clover mix produced butter with a higher proportion of conjugated linoleic acid than butter produced from total mixed ration milk and produced butter with lower hardness. The diet composition could affect the butter characteristic by affecting

the fatty acid composition of milk and beneficial fatty acid composition in terms of butter can be achieved through manipulation of the diet components of cows.

As the review reports, breed, genetic variability, stage of lactation, energy balance, and diet are important factors affecting the fatty acid composition and processability of milk. Another important factor would be milking frequency. Delamaire and Guinard-Flament [140] studied the effect of different milking intervals (8, 12, 16, and 24 hours) on milk fatty acid composition and nutrient uptake by mammary glands in 4 F cows in early lactation for 7 days using a Latin square design. The authors reported that the proportions of SCFA decreased linearly, MCFA did not change, C16 fatty acids were higher with 12- and 24-hour milking intervals, and C18 fatty acids varied cubically with maximum with 6- and 16-hour milking intervals. The authors also reported that the reason for production loss is reduced nutrient extraction by the mammary gland. The extraction of  $\beta$ -hydroxybutyrate, a precursor for fatty acid synthesis, was lower when extending the milking interval whereas the extraction of acetate, another precursor for fatty acid synthesis, was constant with changing milking intervals. Similarly, Wilde and Knight [141] reported a reduction in the activity of enzymes acetyl-CoA-carboxylase and fatty acid synthase in goats milked OAD for 4.5 weeks. Farr et al. [142] reported a decrease in the activity of mammary enzymes in J cows milked OAD for 3 weeks. Longer milking intervals caused the variation in the regulation of nutrient extraction by the mammary gland [140] which could be a reason for lower milk yield and changes in the gross milk composition and fatty acid composition. However, a strong conclusion cannot be derived as the above study reports the effect of milking frequency on fatty acid composition for a short period with a few numbers of cows. The effect of OAD milking on fatty acid composition could vary when cows are milked for full lactation and the results would be more accurate when the sample size is higher. Determination of milk composition and fatty acid composition of OAD milk would help in studying the suitability of OAD milk for milk processing especially, cheese and butter manufacture.

## **2.5. Genome-wide association studies for processability traits**

Investigating the genetic architecture of important traits provides an advantage in selecting animals in their early stage and improves genetic gain for these traits. Genome-wide association studies (GWAS) identify the underlying genetic variants in the genome of animals. The availability of a large number of single nucleotide polymorphisms (SNPs)

provides opportunities to identify possible genetic variation for traits through GWAS [143]. Several studies have conducted GWAS for milk production traits and milk composition traits [144-146]. A better understanding of quantitative trait loci associated with the processability characteristics of milk would be beneficial for the dairy industry.

### **2.5.1. Genome-wide association studies for cheese and milk coagulation properties**

Genomic studies to identify chromosomal regions associated with MCPs and cheese-making characteristics would help in the selection of individuals for breeding programmes. A genome-wide association study by Dadousis et al. [147] reported chromosomes 13 and 15 were associated with RCT, chromosome 6 with K<sub>20</sub>, and chromosome 19 with A<sub>30</sub>, in Brown Swiss cows. The authors also reported associations between MCPs and chromosomes 1, 9, 11, 16, 20, and 28. Tyrisevä et al. [148] found associations between chromosomes 13, 15, and 16 and MCPs in Finnish Ayrshire cows. Sanchez et al. [149] reported that the DGAT1 gene on chromosome 14 was associated with milk composition and MCPs in Montbéliarde cows. Similarly, other studies by Dadousis et al. [150]; Marina et al. [151] reported genome-wide association studies for MCPs. Incorporating this genomic information in the breeding programme could lead to rapid genetic gain for MCPs and cheese-making characteristics.

### **2.5.2. Genome-wide association studies for butter and fatty acids**

Genetic variations in fatty acid composition have been extensively studied [152-154]. Several studies also have performed GWAS to identify chromosomal regions associated with fatty acid composition in milk. In Dutch Friesian cows, Stoop et al. [155] reported significant associations between chromosomes 14 and 26 and MCFA, and an association between chromosome 26 and MUFA. In the same breed, Schennink et al. [156] reported associations between chromosome 14 and total SFA, UFA, and C18 *cis* unsaturated fatty acids, chromosome 15 and C18:1 trans fatty acid, and chromosome 16 and C18:0 and conjugated linolic acid. In Norwegian red cows, Olsen et al. [157] reported an association between chromosome 13 and *de novo* fatty acids, and Knutson et al. [158] reported associations for C16:0 on chromosomes 11, 16 and 27, and for C18:1 *cis*-9 on chromosomes 5,13 and 19. Palombo et al. [159] reported GWAS revealed signals on chromosomes 19 and 26 for milk fatty acids in Italian Simmental and Italian Holstein cows. In New Zealand, Tiplady et al. [160] reported significant effects on chromosome 17 for SCFA, chromosomes 11,19 and 26 for MCFA, and chromosome 14 for LCFA in

F×J cows. The identification of chromosomes and genes and the estimation of genetic parameters confirm the presence of genetic variability for fatty acids which could be used in selective breeding to modify the processing properties and nutritional value of milk. Similarly, conducting genome-wide association studies would help to identify the chromosomal regions responsible for butter characteristics and help to select individuals for breeding programmes.

There has been no research investigating the genome-wide association for the properties of butter. However, the GWAS for fatty acids explains the importance of exploring the genetic basis the of processability characteristics of milk in terms of butter. Stoop et al. [155] reported that GWAS would be useful in selecting animals producing milk with a lower proportion of C16:0 which could reduce the melting point of milk fat and increase the spreadability of butter.

## **2.6. Conclusions and gaps in knowledge**

The dairy industry plays an important role in New Zealand's economy. Milk and milk solids production in New Zealand continues to increase with several dairy products being exported to other countries. Milk composition plays an important role in milk processability. Milk fat, especially fatty acids, are the major components that influence the functional properties of dairy products. As discussed in this review, milk fatty acids are affected by several factors including breed, genetic variability, stage of lactation, diet, and energy balance. The milking frequency could be one of the factors and its effect has not yet been investigated.

The effect of OAD milking on percentages of fat, protein, and lactose, and protein composition was extensively studied in New Zealand [21,24]. In addition, the effect of OAD milk on the processability of milk, mainly cheese making, has also been greatly studied in terms of protein composition [30,32,54]. However, the effect of OAD milking on fatty acid composition has not yet been researched and therefore, the effect of OAD milking on the processability of milk in terms of fatty acids is not yet known.

The fatty acid composition of milk becomes important when considering dairy products, for example, butter as it affects the composition, spreadability/hardness, and melting point [103,129].

The current study investigated:

1. the effect of OAD milking on the fatty acid composition of milk at different stages of lactation compared with TAD milking (Chapter 3).
2. the effect of breed on the fatty acid composition of cows milked OAD and TAD at different stages of lactation (Chapter 4).
3. the association of SNP rs109421300 with the fatty acid composition and processability of milk from cows milked OAD and TAD (Chapter 5).
4. milk composition and milk coagulation properties of different breeds milked OAD and TAD morning and afternoon (Chapter 6).

## References

1. DCANZ. 2000. Dairy's economic contribution, 2020 update. [https://www.dcanz.com/UserFiles/DCANZ/File/Dairy%20economic%20contribution%20slides%20\\_Sense%20Partners%20August%202020.pdf](https://www.dcanz.com/UserFiles/DCANZ/File/Dairy%20economic%20contribution%20slides%20_Sense%20Partners%20August%202020.pdf) Accessed on 22nd December 2022.
2. TDB Advisory. 2020. The Dairy Sector in New Zealand. <https://www.productivity.govt.nz/assets/Inquiries/frontier-firms/a977484e51/The-dairy-sector-in-NZ-TDB-Advisory.pdf> Accessed on 21<sup>th</sup> December 2022.
3. LIC and DairyNZ. 2021. New Zealand Dairy Statistics 2020-21. <https://www.dairynz.co.nz/media/5794941/nz-dairy-statistics-2020-21-web.pdf> Accessed on 21<sup>th</sup> December 2022.
4. DCANZ. 2020. About the NZ Dairy Industry. <https://www.dcanz.com/about-the-nz-dairy-industry/> Accessed on 13th December 2023.
5. Back, P. Dairy production. In *Livestock production in New Zealand*. Stafford, K., Eds.; Massey University Press: Palmerston North, New Zealand, 2017; pp. 14–53.
6. Holmes CW, Brookes IM, Garrick DJ, Mackenzie DDS, Parkinson TJ, Wilson GF. Pastoral dairy farming in New Zealand. In *Milk Production from Pasture: Principles and Practices*, Swain, D.; Eds.; Massey University, Palmerston North, New Zealand, 2002; pp. 21-31.
7. Whitehead, D.C. 1995. *Grassland Nitrogen*. CABI, Wallingford, UK.
8. Wales, W.J.; Kolver, E.S. Challenges of feeding dairy cows in Australia and New Zealand. *Anim. Prod. Sci.* **2017**, *57*, 1366-1383. <https://doi.org/10.1071/AN16828>

9. DairyNZ. 2016a. Breeding Worth. <https://www.dairynz.co.nz/animal/animal-evaluation/interpreting-the-info/breeding-worth/> Accessed on 22nd December 2022.
10. Harris B.L. Breeding dairy cows for the future in New Zealand. *N Z Vet J.* **2005**, 53, 384-389. <https://doi.org/10.1080/00480169.2005.36582>
11. DairyNZ. 2020. Milking intervals. <https://www.dairynz.co.nz/milking/milking-intervals/> Accessed on 22nd December 2022.
12. DairyNZ. 2016b. Once a day milking. <https://www.dairynz.co.nz/milking/milking-intervals/once-a-day-milking/> Accessed on 14th December 2022.
13. Davis, S.R.; Farr, V.C.; Stelwagen, K. Regulation of yield loss and milk composition during once-daily milking: A review. *Livest. Prod. Sci.* **1999**, 59, 77–94, [https://doi.org/10.1016/S0301-6226\(98\)00204-8](https://doi.org/10.1016/S0301-6226(98)00204-8)
14. Phyn, C.V.C.; Kay, J.K.; Rius, A.G.; Morgan, S.R.; Roach, C.S.; Grala, T.M.; Roche, J.R. Effect of temporary alterations to milking frequency during the early post-partum period on milk production and body condition score in grazing dairy cows. *Proc. N. Z. Soc. Anim. Prod.* **2011**, 71, 45–49.
15. Kay, J.K.; Phyn, C.V.; Rius, A.G.; Morgan, S.R.; Grala, T.M.; Roche, J.R. Once-daily milking during a feed deficit improves energy status in early lactating dairy cows. *Proc. N. Z. Soc. Anim. Prod.* **2012**, 72, 129-133.
16. Stockdale, C.R. Influence of milking frequency on the productivity of dairy cows. *Aust. J. Exp. Agric.* **2006**, 46, 965-794. <http://dx.doi.org/10.3168/jds.2013-6764>
17. Edwards, J.P. Comparison of milk production and herd characteristics in New Zealand herds milked once or twice a day. *Anim. Prod. Sci.* **2019**, 59, 570–580. <https://doi.org/10.1071/AN17484>
18. Bewsell, D.; Clark, D.A.; Dalley, D.E. Understanding motivations to adopt once-a-day milking amongst New Zealand dairy farmers. *J. Agric. Educ. Ext.* **2008**, 14, 69–80. <https://doi.org/10.1080/13892240701820611>
19. Gleeson, D.E.; O'Brien, B.; Boyle, L.; Earley, B. Effect of milking frequency and nutritional level on aspects of the health and welfare of dairy cows. *Animal.* **2007**, 1, 125-132. <https://doi.org/10.1017/S1751731107658030>
20. Kendall, P.E.; Tucker, C.B.; Dalley, D.E.; Clark, D.A.; Webster, J.R. Milking frequency affects the circadian body temperature rhythm in dairy cows. *Livest. Sci.* **2008**, 117, 130-138. <https://doi.org/10.1016/j.livsci.2007.12.009>

21. Rémond, B.; Coulon, J.B.; Nicloux, M.; Levieux, D. Effect of temporary once-daily milking in early lactation on milk production and nutritional status of dairy cows. *Ann. Zootech.* **1999**, *48*, 341–352, <https://hal.archives-ouvertes.fr/hal-00889807>
22. Clark, D.A.; Phyn, C.V.C.; Tong, M.J.; Collis, S.J.; Dalley, D.E. A systems comparison of once-versus twice daily milking of pastured dairy cows. *J. Dairy Sci.* **2006**, *89*, 1854–1862. [https://doi.org/10.3168/jds.S0022-0302\(06\)72254-8](https://doi.org/10.3168/jds.S0022-0302(06)72254-8)
23. Rémond, B.; Pomiès, D.; Dupont, D.; Chilliard, Y. Once-a-day milking of multiparous Holstein cows throughout the entire lactation: Milk yield and composition, and nutritional status. *Anim. Res.* **2004**, *53*, 201–212, <https://doi.org/10.1051/animres:2004014>
24. Holmes, C.W.; Wilson, G.F.; Mackenzie, D.D.; Purchas, J. The effects of milking once-daily throughout lactation on the performance of dairy cows grazing on pasture. *Proc. N. Z. Soc. Anim. Prod.* **1992**, *52*, 13-16.
25. Tong, M.J.; Clark, D.A.; Cooper, C.V. Once-a-day milking: Possible and profitable? *Proc. N. Z. Grassl. Assoc.* **2002**, *64*, 33–37, <https://doi.org/10.33584/jnzs.2002.64.2470>
26. Stelwagen, K.; Phyn, C.V.V.; Davis, S.R.; Guinard-Flament, J.; Pomiès, D.; Roche, J.R.; Kay, J.K. Invited review: Reduced milking frequency. Milk production and management implications. *J. Dairy Sci.* **2013**, *96*, 1–13, <https://doi.org/10.3168/jds.2012-6074>
27. Claesson, O.; Hansson, A.; Gustafsson, N.; Brannang, E. Studies on monozygous cattle twins. XVII. Once-a-day milking compared with twice-a-day milking. *Acta Agriculturae Scandinavica B.* **1959**, *9*, 38–58. <https://doi.org/10.1080/00015125909433307>
28. Carruthers, V.R.; Davis, S.R.; Bryant, A.M.; Henderson, H.V.; Morris, C.A.; Copeman, P.J. Response of Jersey and Friesian cows to once a day milking and prediction of response based on udder characteristics and milk composition. *J. Dairy Sci.* **1993**, *60*, 1–11, <https://doi.org/10.1017/S0022029900027291>
29. Knutson, R.J.; MacKenzie, D.D.; Davis, S.R.; McCutcheon, S.N. The effect of once daily milking on concentrations and yields of plasminogen, plasmin and other whey proteins. *Proc. N. Z. Soc. Anim. Prod.* **1993**, *53*, 155-158.
30. Stelwagen, K.; Davis, S.R.; Farr, V.C.; Eichler, S.J.; Politis, I. Effect of once daily milking and concurrent somatotropin on mammary tight junction permeability

- and yield of cows. *J. Dairy Sci.* **1994**, *77*, 2994-3001.  
[https://doi.org/10.3168/jds.S0022-0302\(94\)77240-4](https://doi.org/10.3168/jds.S0022-0302(94)77240-4)
31. Auldish, M.J.; Prosser, C.G. Differential effects of short-term once-daily milking on milk yield, milk composition and concentrations of selected blood metabolites in cows with low or high pasture intake. *Proc. N. Z. Soc. Anim. Prod.* **1998**, *58*, 41-43.
32. O'Brien, B.; Ryan, G.; Meaney, W.J.; McDonagh, D.; Kelly, A. Effect of frequency of milking on yield, composition, and processing quality of milk. *J. Dairy Res.* **2002**, *69*, 367-374, <https://doi.org/10.1017/S0022029902005605>
33. Rémond, B.; Aubailly, S.; Chilliard, Y.; Dupont, D.; Pomiès, D.; Petit, M. Combined effects of once-daily milking and feeding level in the first three weeks of lactation on milk production and enzyme activities, and nutritional status, in Holstein cows. *Anim. Res.* **2002**, *51*, 101-117.  
<https://doi.org/10.1051/animres:2002014>
34. Pomiès, D.; Martin, B.; Chilliard, Y.; Pradel, P.; Rémond, B. Once-a-day milking of Holstein and Montbéliarde cows for 7 weeks in mid-lactation. *Animal* **2007**, *1*, 1497-1505, <https://doi.org/10.1017/S1751731107000778>
35. Ferris, C.P.; Frost, J.P.; Mayne, C.S.; McCoy, M.A.; Kilpatrick, D.J. A comparison of the direct and residual response of dairy cows to once or twice-daily milking, in late lactation. *Livest. Sci.* **2008**, *114*, 305-314,  
<https://doi.org/10.1016/j.livsci.2007.05.015>
36. McNamara, S.; Murphy, J.J.; O'mara, F.P.; Rath, M.; Mee, J.F. Effect of milking frequency in early lactation on energy metabolism, milk production and reproductive performance of dairy cows. *Livest. Sci.* **2008**, *117*, 70-78.  
[https://doi.org/10.3168/jds.S0022-0302\(06\)72215-9](https://doi.org/10.3168/jds.S0022-0302(06)72215-9)
37. Kay, J.K.; Phyn, C.V.; Rius, A.G.; Morgan, S.R.; Grala, T.M.; Roche, J.R. Effect of milking frequency and nutrition in early lactation on milk production and body condition in grazing dairy cows. *Proc. N. Z. Soc. Anim. Prod.* **2011**, *71*, 37-41.
38. Grala, T.M.; Phyn, C.V.; Kay, J.K.; Rius, A.G.; Littlejohn, M.D.; Snell, R.G.; Roche, J.R. Temporary alterations to milking frequency, immediately post-calving, modified the expression of genes regulating milk synthesis and apoptosis in the bovine mammary gland. *Proc. N. Z. Soc. Anim. Prod.* **2011**, *71*, 3-8

39. Wilde, C.J.; Knight, C.H. Milk yield and mammary function in goats during and after once-daily milking. *J. Dairy Res.* **1990**, *57*, 441–447. <https://doi.org/10.1017/S0022029900029484>
40. Littlejohn, M.D.; Walker, C.G.; Ward, H.E.; Lehnert, K.B.; Snell, R.G.; Verkerk, G.A.; Spelman, R.J.; Clark, D.A.; Davis, S.R. Effects of reduced frequency of milk removal on gene expression in the bovine mammary gland. *Physiol. Genomics.* **2010**, *41*, 21–32. <https://doi.org/10.1152/physiolgenomics.00108.2009>
41. Grala, T.M.; Phyn, C.V.; Kay, J.K.; Rius, A.G.; Littlejohn, M.D.; Snell, R.G.; Roche, J.R. Temporary alterations to milking frequency, immediately post-calving, modified the expression of genes regulating milk synthesis and apoptosis in the bovine mammary gland. *Proc. N. Z. Soc. Anim. Prod.* **2011**, *71*, 3–8
42. Stelwagen, K.; Prosser, C.G.; Davis, S.R.; Farr, V.C.; Politis, I.; White, J.H.; Zavizion, B. Effect of milking frequency and somatotropin on the activity of plasminogen activator, plasminogen, and plasmin in bovine milk. *J. Dairy Sci.* **1994**, *77*, 3577–3583. [https://doi.org/10.3168/jds.S0022-0302\(94\)77301-X](https://doi.org/10.3168/jds.S0022-0302(94)77301-X)
43. Lucey, J.A.; Hauth, B.; Gorry, C.; Fox, P.F. The acid-base buffering properties of milk. *Milchwissenschaft*, **1993**, *48*, 268–272.
44. Walstra, P. On the stability of casein micelles. *J. Dairy Sci.* **1990**, *73*, 1965–1979. [https://doi.org/10.3168/jds.S0022-0302\(90\)78875-3](https://doi.org/10.3168/jds.S0022-0302(90)78875-3)
45. McMahon, D.J.; Brown, R.J. 1984. Enzymic coagulation of casein micelles: a review. *J. Dairy Sci.* **1984**, *67*, 919–29. [https://doi.org/10.3168/jds.S0022-0302\(84\)81390-9](https://doi.org/10.3168/jds.S0022-0302(84)81390-9)
46. Summer, A.; Malacarne, M.; Martuzzi, F.; Mariani, P. Structural and functional characteristics of Modenese cow milk in Parmigiano-Reggiano cheese production. *Ann. Fac. Med. Vet. Univ. Parma.* **2002**, *22*, 163–174.
47. Ikonen, T. 2000. Possibilities of genetic improvement of milk coagulation properties of dairy cows. Academic diss. Univ. Helsinki, Finland.
48. Ikonen, T., Ruottinen, O., Syväoja, E.L. Effect of milk coagulation properties of herd bulk milks on yield and composition of Emmental cheese. *Agric. Food Sci.* **1999**, *8*, 411–422. <https://doi.org/10.23986/afsci.5638>
49. Malacarne, M.; Summer, A.; Fossa, E.; Formaggioni, P.; Franceschi, P.; Pecorari, M.; Mariani, P. Composition, coagulation properties and Parmigiano-Reggiano cheese yield of Italian Brown and Italian Friesian herd milks. *J. Dairy Res.* **2006**, *73*, 171–177. <https://doi.org/10.1017/S0022029905001688>

50. Cecchinato, A.; Bittante, G. Genetic and environmental relationships of different measures of individual cheese yield and curd nutrients recovery with coagulation properties of bovine milk. *J. Dairy Sci.* **2016**, *99*, 1975-1989. <https://doi.org/10.3168/jds.2015-9629>
51. Auldust, M.J.; Johnston, K.A.; White, N.J.; Fitzsimons, W.P.; Boland, M.J. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *J. Dairy Sci.* **2004**, *71*, 51–57, <https://doi.org/10.1017/s0022029903006575>
52. Jensen, H.B.; Poulsen, N.A.; Andersen, K.K.; Hammershøj, M.; Poulsen, H.D.; Larsen, L.B. Distinct composition of bovine milk from Jersey and Holstein-Friesian cows with good, poor, or noncoagulation properties as reflected in protein genetic variants and isoforms. *J. Dairy Sci.* **2012**, *95*, 6905-6917. <https://doi.org/10.3168/jds.2012-5675>
53. Stocco, G.; Cipolat-Gotet, C.; Bobbo, T.; Cecchinato, A.; Bittante, G. Breed of cow and herd productivity affect milk composition and modeling of coagulation, curd firming, and syneresis. *J. Dairy Sci.* **2017**, *100*, 129-145. <https://doi.org/10.3168/jds.2016-11662>
54. De Marchi, M.; Dal Zotto, R.; Cassandro, M.; Bittante, G. Milk coagulation ability of five dairy cattle breeds. *J. Dairy Sci.* **2007**, *90*, 3986–3992. <https://doi.org/10.3168/jds.2006-627>.
55. Martin, B.; Pomiès, D.; Pradel, P.; Verdier-Metz, I.; Rémond, B. Yield and sensory properties of cheese made with milk from Holstein or Montbéliarde cows milked twice or once daily. *J. Dairy Sci.* **2009**, *92*, 4730–4737. <https://doi.org/10.3168/jds.2008-1914>
56. De Marchi, M.; Bittante, G.; Dal Zotto, R.; Dalvit, C.; Cassandro, M. Effect of Holstein Friesian and Brown Swiss breeds on quality of milk and cheese. *J. Dairy Sci.* **2008**, *91*, 4092-4102. <https://doi.org/10.3168/jds.2007-0788>
57. Franceschi, P.; Malacarne, M.; Formaggioni, P.; Faccia, M.; Summer, A. Quantification of the effect of the cattle breed on milk cheese yield: Comparison between Italian Brown Swiss and Italian Friesian. *Animals*. **2020**, *10*, 1331. <https://doi.org/10.3390/ani10081331>.
58. Jõudu, I.; Henno, M.; Kaart, T.; Püssa, T.; Kärt, O. The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. *Int. Dairy J.* **2008**, *18*, 964-967. <https://doi.org/10.1016/j.idairyj.2008.02.002>

59. Amenu, B.; Deeth, H.C. The impact of milk composition on cheddar cheese manufacture. *Aust. J. Dairy Technol.* **2007**, *62*, 171–184.
60. Amalfitano, N.; Cipolat-Gotet, C.; Cecchinato, A.; Malacarne, M.; Summer, A.; Bittante, G. Milk protein fractions strongly affect the patterns of coagulation, curd firming, and syneresis. *J. Dairy Sci.* **2019**, *102*, 2903–2917. <https://doi.org/10.3168/jds.2018-15524>
61. Kübarsepp, I.; Henno, M.; Viinalass, H.; Sabre, D. Effect of  $\kappa$ -casein and  $\beta$ -lactoglobulin genotypes on the milk rennet coagulation properties. *Agric. Res.* **2005**, *3*, 55–64.
62. Nájera, A.I.; De Renobales, M.; Barron, L.J. Effects of pH, temperature, CaCl<sub>2</sub> and enzyme concentrations on the rennet-clotting properties of milk: a multifactorial study. *Food Chem.* **2003**, *80*, 345–352. [https://doi.org/10.1016/S0308-8146\(02\)00270-4](https://doi.org/10.1016/S0308-8146(02)00270-4)
63. Ikonen, T.; Morri, A.; Tyrisevä, A.-M.; Ruottinen, O.; Ojala, M. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content and pH of milk. *J. Dairy Sci.* **2004**, *87*, 458–467. [https://doi.org/10.3168/jds.S0022-0302\(04\)73185-9](https://doi.org/10.3168/jds.S0022-0302(04)73185-9)
64. De Marchi, M.; Fagan, C.C.; O'Donnell, C.P.; Cecchinato, A.; Dal Zotto, R.; Cassandro, M.; Penasa, M.; Bittante, G. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. *J. Dairy Sci.* **2009**, *92*, 423–432. <https://doi.org/10.3168/jds.2008-1163>
65. Ketto, I.A.; Knutsen, T.M.; Øyaas, J.; Heringstad, B.; Ådnøy, T.; Devold, T.G.; Skeie S.B. Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. *Int. Dairy J.* **2017**, *70*, 55–64. <https://doi.org/10.1016/j.idairyj.2016.10.010>.
66. Stocco, G.; Summer, A.; Cipolat-Gotet, C.; Malacarne, M.; Cecchinato, A.; Amalfitano, N.; Bittante, G. The mineral profile affects the coagulation pattern and cheese-making efficiency of bovine milk. *J. Dairy Sci.* **2021**, *104*, 8439–8453. <https://doi.org/10.3168/jds.2021-20233>
67. Auldish, M.J.; Walsh, B.J.; Thomson, N.A. Seasonal and lactational influences on bovine milk composition in New Zealand. *J. Dairy Res.* **1998**, *65*, 401–411, <https://doi.org/10.1017/s0022029998002970>

68. Edwards, J.P.; Jago, J.G.; Lopez-Villalobos, N. Analysis of milking characteristics in New Zealand dairy cows. *J. Dairy Sci.* **2014**, *97*, 259-69. <https://doi.org/10.3168/jds.2013-7051>
69. Gulati, A.; Galvin, N.; Hennessy, D.; McAuliffe, S.; O'Donovan, M.; McManus, J.J.; Fenelon, M.A.; Guinee, T.P. Grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on low-moisture part-skim Mozzarella cheese yield and quality characteristics in mid and late lactation. *J. Dairy Sci.* **2018**, *101*, 8737-8756. <https://doi.org/10.3168/jds.2018-14566>
70. Auldust, M.J.; Coats, S.; Sutherland, B.J.; Mayes, J.J.; McDowell, G.H.; Rogers, G.L. Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of Cheddar cheese. *J. Dairy Res.* **1996**, *63*, 269-280. <https://doi.org/10.1017/S0022029900031769>
71. Albenzio, M.; Caroprese, M.; Santillo, A.; Marino, R.O.; Taibi, L.; Sevi, A. Effects of somatic cell count and stage of lactation on the plasmin activity and cheese-making properties of ewe milk. *J. Dairy Sci.* **2004**, *87*, 533-542. [https://doi.org/10.3168/jds.S0022-0302\(04\)73194-X](https://doi.org/10.3168/jds.S0022-0302(04)73194-X)
72. Coulon, J.B.; Verdier, I.; Pradel, P.; Almena, M. Effect of lactation stage on the cheesemaking properties of milk and the quality of Saint-Nectaire-type cheese. *J. Dairy Res.* **1998**, *65*, 295-305. <https://doi.org/10.1017/S002202999700277X>
73. Ostersen, S.; Foldager, J.; Hermansen, J.E. Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. *J. Dairy Res.* **1997**, *64*, 207-219. <https://doi.org/10.1017/S0022029996002099>
74. Palmquist, D.L.; Beaulieu, A.D.; Barbano, D.M. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* **1993**, *76*, 1753-1771, [https://doi.org/10.3168/jds.S0022-0302\(93\)77508-6](https://doi.org/10.3168/jds.S0022-0302(93)77508-6)
75. Hurtaud, C.; Peyraud, J.L.; Michel, G.; Berthelot, D.; Delaby, L. Winter feeding systems and dairy cow breed have an impact on milk composition and flavour of two Protected Designation of Origin French cheeses. *Animal*. **2009**, *3*, 1327-1338. <https://doi.org/10.1017/S1751731109004716>
76. Coakley, M.; Barrett, E.; Murphy, J.J.; Ross, R.P.; Devery, R.; Stanton C. Cheese manufacture with milk with elevated conjugated linoleic acid levels caused by dietary manipulation. *J. Dairy Sci.* **2007**, *90*, 2919-2927. <https://doi.org/10.3168/jds.2006-584>

77. Carpino, S.; Mallia, S.; La Terra, S.; Melilli, C.; Licitra, G.; Acree, T.E.; Barbano, D.M.; Van Soest, P.J. Composition and aroma compounds of Ragusano cheese: native pasture and total mixed rations. *J. Dairy Sci.* **2004**, *87*, 816-830. [https://doi.org/10.3168/jds.S0022-0302\(04\)73226-9](https://doi.org/10.3168/jds.S0022-0302(04)73226-9)
78. O'Callaghan, T.F.; Mannion D.T.; Hennessy, D.; McAuliffe, S.; O'Sullivan, M.G.; Leeuwendaal, N.; Beresford, T.P.; Dillon, P.; Kilcawley, K.N.; Sheehan, J.J.; Ross, R.P. Effect of pasture versus indoor feeding systems on quality characteristics, nutritional composition, and sensory and volatile properties of full-fat Cheddar cheese. *J. Dairy Sci.* **2017**, *100*, 6053-73. <https://doi.org/10.3168/jds.2016-12508>
79. Lacy-Hulbert, S.J.; Woolford, M.W.; Nicholas, G.D.; Prosser, C.G.; Stelwagen, K. Effect of milking frequency and pasture intake on milk yield and composition of late lactation cows. *J. Dairy Sci.* **1999**, *82*, 1232-1239. [https://doi.org/10.3168/jds.S0022-0302\(99\)75346-4](https://doi.org/10.3168/jds.S0022-0302(99)75346-4)
80. Stelwagen, K.; Van Espen, D.C.; Verkerk, G.A.; McFadden, H.A.; Farr, V.C. Elevated plasma cortisol reduces permeability of mammary tight junctions in the lactating bovine mammary epithelium. *J. Endocrinol.* **1998**, *159*, 173-178. <https://doi.org/10.1677/joe.0.1590173>
81. Mona, A.M.; El-Gawad, A.; Nawal, S.A. Cheese yield as affected by some parameters. Review. *Acta Sci. Pol., Technol. Aliment.* **2011**, *10*, 131-53.
82. Jensen, R.G. The composition of bovine milk lipids. *J. Dairy Sci.* **2002**, *85*, 295-350. [https://doi.org/10.3168/jds.S0022-0302\(02\)74079-4](https://doi.org/10.3168/jds.S0022-0302(02)74079-4)
83. MacGibbon, A.K.H. Composition and Structure of Bovine Milk Lipids. In *Advanced Dairy Chemistry*, 4th ed.; McSweeney, P.L.H., Fox, P.F., O'Mahony, J.A., Eds.; Springer: New York, NY, USA, 2020; Volume 2, pp. 1–32.
84. Jensen, R.G.; Newburg, D.S. Bovine milk lipids. In *handbook of milk composition*, 1st ed.; Jensen, R.G; Eds.; Academic Press: USA, 1995; 543-575.
85. Grummer, R.R. Effect of feed on the composition of milk fat. *J. Dairy Sci.* **1991**, *74*, 3244–3257. [https://doi.org/10.3168/jds.S0022-0302\(91\)78510-X](https://doi.org/10.3168/jds.S0022-0302(91)78510-X)
86. Shingfield, K.J.; Ahvenjärvi, S.; Toivonen, V.; Ärölä, A.; Nurmela, K.V.; Huhtanen, P.; Griinari, J.M. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Anim. Sci.* **2003**, *77*, 165-179. <https://doi.org/10.1017/S1357729800053765>

87. Ratnayake, W.N.; Galli, C. Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism. *Ann. Nutr. Metab.* **2009**, *55*, 8-43. <https://doi.org/10.1159/000228994>
88. Huth, P.J. Ruminant trans fatty acids: composition and nutritional characteristics. In *Trans Fats in Foods*, List, G.R.; Kristchevsky, D.; Ratnayake, W.M.N.; Eds.; Urbana, AOCS Press, 2007; pp 97–126.
89. Kepler, C.R.; Hirons, K.P.; McNeill, J.J.; Tove, S.B. Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. *J. Biol. Chem.* **1966**, *24*, 1350-1354. [https://doi.org/10.1016/S0021-9258\(18\)96781-5](https://doi.org/10.1016/S0021-9258(18)96781-5)
90. Palmquist, D.L. Milk fat: in Origin of fatty acids and influence of nutritional factors thereon. In *Advanced dairy chemistry*, 3rd ed.; Fox, P.F.; McSweeney, P.L.H.; Eds.; Springer: Boston, MA, 2006; volume 2, pp. 43-92. [https://doi.org/10.1007/0-387-28813-9\\_2](https://doi.org/10.1007/0-387-28813-9_2)
91. Månsson, H.L. Fatty acids in bovine milk fat. *Food Nutr Res.* **2008**, *52*. doi: 10.3402/fnr.v52i0.1821.
92. Yahyaoui, M.H.; Sanchez, A.; Folch, J.M. Rapid communication: Partial nucleotide sequence of the goat stearyl coenzyme A desaturase cDNA and gene structure. *J Anim Sci.* **2002**, *80*, 866-867. <https://doi.org/10.2527/2002.803866x>
93. Yen, C.L.; Stone, S.J.; Koliwad, S.; Harris, C.; Farese, R.V. Thematic review series: Glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *J. Lipid Res.* **2008**, *49*, 2283-2282. <https://doi.org/10.1194/jlr.R800018-JLR200>
94. Gimeno, R.E.; Cao, J. Thematic review series: glycerolipids. Mammalian glycerol-3-phosphate acyltransferases: new genes for an old activity. *J. Lipid Res.* **2008**, *49*, 2079-2088. <https://doi.org/10.1194/jlr.R800013-JLR200>
95. Turchetto-Zolet, A.C.; Maraschin, F.S.; de Morais, G.L.; Cagliari, A.; Andrade, C.; Margis-Pinheiro, M.; Margis, R. Evolutionary view of acyl-CoA diacylglycerol acyltransferase (DGAT), a key enzyme in neutral lipid biosynthesis. *BMC Evol. Biol.* **2011**, *11*, 1-14. <https://doi.org/10.1186/1471-2148-11-263>
96. Cases, S.; Smith, S.J.; Zheng, Y.W.; Myers, H.M.; Lear, S.; Sande, E.; Novak, S.; Collins, C.; Welch, C.B.; Lusic, A.J.; Erickson, S.K. Identification of a gene encoding an acyl CoA: diacylglycerol acyltransferase, a key enzyme in

- triacylglycerol synthesis. *Proc. Natl. Acad. Sci.* **1998**, *95*, 13018-13023. <https://doi.org/10.1073/pnas.95.22.13018>
97. Brunner, J.R. Physical equilibria in milk: The lipid phase. In *Fundamentals of Dairy Chemistry*, 2nd ed.; Webb, B.H.; Johnson, A.H.; Alford, J.A., eds.; Avi Publishing Co: Westport, Conn, 1974; pp. 474- 602
98. Ramaswamy, N.; Baer, R.J.; Schingoethe, D.J.; Hippen, A.R.; Kasperson, K.M.; Whitlock, L.A. Composition and flavor of milk and butter from cows fed fish oil, extruded soybeans, or their combination. *J. Dairy Sci.* **2001**, *84*, 2144–2151. [https://doi.org/10.3168/jds.S0022-0302\(01\)74659-0](https://doi.org/10.3168/jds.S0022-0302(01)74659-0)
99. Gonzalez, S.; Duncan, S.E.; O’Keefe, S.F.; Sumner, S.S.; Herbein, J.H. Oxidation and textural characteristics of butter and ice cream with modified fatty acid profiles. *J. Dairy Sci.* **2003**, *86*, 70-77. [https://doi.org/10.3168/jds.S0022-0302\(03\)73585-1](https://doi.org/10.3168/jds.S0022-0302(03)73585-1)
100. Bobe, G.; Hammond, E.G.; Freeman, A.E.; Lindberg, G.L.; Beitz, D.C. Texture of butter from cows with different milk fatty acid compositions. *J. Dairy Sci.* **2003**, *86*, 3122-3127. [https://doi.org/10.3168/jds.S0022-0302\(03\)73913-7](https://doi.org/10.3168/jds.S0022-0302(03)73913-7)
101. Couvreur, S.; Hurtaud, C.; Lopez, C.; Delaby, L.; Peyraud, J.L. The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties. *J. Dairy Sci.* **2006**, *89*, 1956-1969. [https://doi.org/10.3168/jds.S0022-0302\(06\)72263-9](https://doi.org/10.3168/jds.S0022-0302(06)72263-9)
102. MacGibbon, A.K.; McLennan, W.D. Hardness of New-Zealand patted butter—seasonal and regional variations. *N. Z. J. Dairy Sci. Technol.* **1987**, *22*, 143–156.
103. MacGibbon, A.K.H. Composition and Structure of Bovine Milk Lipids. In *Advanced Dairy Chemistry*, 4th ed.; McSweeney, P.L.H., Fox, P.F., O’Mahony, J.A., Eds.; Springer: New York, NY, USA, 2020; Volume 2, pp. 1–32.
104. Mackle, T.R.; Petch, S.F.; Bryant, A.M.; Auldist, M.J.; Henderson, H.V.; MacGibbon, A.K.H. Variation in the characteristics of milkfat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *N. Z. J. Agric. Res.* **1997**, *40*, 349–359, <https://doi.org/10.1080/00288233.1997.9513254>
105. Avramis, C.A.; Wang, H.; McBride, B.W.; Wright, T.C.; Hill, A.R. Physical and processing properties of milk, butter, and Cheddar cheese from cows fed supplemental fish meal. *J. Dairy Sci.* **2003**, *86*, 2568-2576. [https://doi.org/10.3168/jds.S0022-0302\(03\)73851-X](https://doi.org/10.3168/jds.S0022-0302(03)73851-X)

106. Beaulieu, A.D.; Palmquist, D.L. Differential effects of high-fat diets on fatty acid composition in milk of Jersey and Holstein cows. *J. Dairy Sci.* **1995**, *78*, 1336-1344. [https://doi.org/10.3168/jds.S0022-0302\(95\)76755-8](https://doi.org/10.3168/jds.S0022-0302(95)76755-8)
107. Lopez-Villalobos, N.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Sneddon, N.W.; Holroyd, S.E.; MacGibbon, A.K.; Snell, R.G. Genetic correlations of milk fatty acid contents predicted from milk mid-infrared spectra in New Zealand dairy cattle. *J. Dairy Sci.* **2020**, *103*, 7238-7248. <https://doi.org/10.3168/jds.2019-17971>
108. Soyeurt, H.; Dardenne, P.; Gillon, A.; Croquet, C.; Vanderick, S.; Mayeres, P.; Bertozzi, C.; Gengler, N. Variation in fatty acid contents of milk and milk fat within and across breeds. *J. Dairy Sci.* **2006**, *89*, 4858-4865. [https://doi.org/10.3168/jds.S0022-0302\(06\)72534-6](https://doi.org/10.3168/jds.S0022-0302(06)72534-6).
109. Croissant, A.E.; Washburn, S.P.; Dean, L.L.; Drake, M.A. Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. *J. Dairy Sci.* **2007**, *90*, 4942-4953. <https://doi.org/10.3168/jds.2007-0456>
110. Palladino, R.A.; Buckley, F.; Prendiville, R.; Murphy, J.J.; Callan, J.; Kenny, D.A. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. *J. Dairy Sci.* **2010**, *93*, 2176-2184, <https://doi.org/10.3168/jds.2009-2453>.
111. Soyeurt, H.; Dehareng, F.; Mayeres, P.; Bertozzi, C.; Gengler, N. Variation of  $\Delta 9$ -desaturase activity in dairy cattle. *J. Dairy Sci.* **2008**, *91*, 3211-3224. <https://doi.org/10.3168/jds.2007-0518>
112. Moioli, B.; Contarini, G.; Avalli, A.; Catillo, G.; Orru, L.; De Matteis, G.; Masoero, G.; Napolitano, F. Effect of stearoyl-coenzyme A desaturase polymorphism on fatty acid composition of milk. *J. Dairy Sci.* **2007**, *90*, 3553-3558. <https://doi.org/10.3168/jds.2006-855>
113. Maurice-Van Eijndhoven, M.H.; Bovenhuis, H.; Soyeurt, H.; Calus, M.P. Differences in milk fat composition predicted by mid-infrared spectrometry among dairy cattle breeds in the Netherlands. *J. Dairy Sci.* **2013**, *96*, 2570-2582 <https://doi.org/10.3168/jds.2012-5793>
114. Wales, W.J.; Kolver, E.S.; Egan, A.R.; Roche, R. Effects of strain of Holstein-Friesian and concentrate supplementation on the fatty acid composition of milk

- fat of dairy cows grazing pasture in early lactation. *J. Dairy Sci.* **2009**, *92*, 247-255. <https://doi.org/10.3168/jds.2008-1386>
115. Gross, J.; van Dorland, H.A.; Bruckmaier, R.M.; Schwarz, F.J. Milk fatty acid profile related to energy balance in dairy cows. *J. Dairy Sci.* **2011**, *78*, 479–488, <https://doi.org/10.1017/S0022029911000550>
116. Kgwatalala, P.M.; Ibeagha-Awemu, E.M.; Mustafa, A.F.; Zhao, X. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. *Anim. Genet.* **2009**, *40*, 609–615, <https://doi.org/10.1111/j.1365-2052.2009.01887.x>
117. Kay, J.K.; Weber, W.J.; Moore, C.E.; Bauman, D.E.; Hansen, L.B.; Chester-Jones, H.; Crooker, B.A.; Baumgard, L.H. Effects of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. *J. Dairy Sci.* **2005**, *88*, 3886–3893. [https://doi.org/10.3168/jds.S0022-0302\(05\)73074-5](https://doi.org/10.3168/jds.S0022-0302(05)73074-5)
118. Garnsworthy, P.C.; Masson, L.L.; Lock, A.L.; Mottram, T.T. Variation of milk citrate with stage of lactation and *de novo* fatty acid synthesis in dairy cows. *J. Dairy Sci.* **2006**, *89*, 1604–1612. [https://doi.org/10.3168/jds.S0022-0302\(06\)72227-5](https://doi.org/10.3168/jds.S0022-0302(06)72227-5)
119. Bilal, G.; Cue, R.I.; Mustafa, A.F.; Hayes, J.F. Effects of parity, age at calving and stage of lactation on fatty acid composition of milk in Canadian Holsteins. *Can. J. Anim. Sci.* **2014**, *94*, 401–410. <https://doi.org/10.4141/cjas2013-172>
120. Schwendel, B.H.; Morel, P.C.; Wester, T.J.; Tavendale, M.H.; Deadman, C.; Fong, B.; Shadbolt, N.M.; Thatcher, A.; Otter, D.E. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. *J. Dairy Sci.* **2015**, *98*, 1411–1425, <https://doi.org/10.3168/jds.2014-8322>
121. Churakov, M.; Karlsson, J.; Rasmussen, A.E.; Holtenius K. Milk fatty acids as indicators of negative energy balance of dairy cows in early lactation. *Animal*, **2021**, *15*, 100253. <https://doi.org/10.1016/j.animal.2021.100253>
122. Stoop, W.M.; Bovenhuis, H.; Heck, J.M.L.; van Arendonk, J.A.M. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *J. Dairy Sci.* **2009**, *92*, 1469–1478. <https://doi.org/10.3168/jds.2008-1468>.
123. Thomson, N.A.; Poel, W. Seasonal variation of the fatty acid composition of milkfat from Friesian cows grazing pasture. *Proc. N. Z. Soc. Anim. Prod.* **2000**, *60*, 314–317.

124. Silva-Villacorta, D.; Lopez-Villalobos, N.; Blair, H.T.; Hickson, R.E.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Snell, R.G. Effect of lactation stage and breed on the concentration of unsaturated fatty acids in milkfat of New Zealand dairy cattle. *Proc. N. Z. Soc. Anim. Prod.* **2011**, *71*, 203-207.
125. Bauman, D.E.; Davis, C.L. Biosynthesis of milk fat. In *Lactation-A Comprehensive Treatise*. Larson, B.L. and Smith, V.R., Eds.; Academic Press: New York, NY. 1974; Volume 2, pp. 31-75.
126. Bauman, D.E.; Lock, A.L.; Corl, B.A.; Ip, C.; Salter, A.M.; Parodi, P.W. Milk fatty acids and human health: potential role of conjugated linoleic acid and trans fatty acids. In *Ruminant physiology: Digestion, metabolism and impact of nutrition on gene expression, immunology and stress*, Wageningen Academic Publishers, Wageningen, the Netherlands, 2006. (pp. 523-555).
127. Şanta, A.; Mierlita, D.; Dărăban, S.; Socol, C.T.; Vicas, S.I.; Şuteu, M.; Maerescu, C.M.; Stanciu, A.S.; Pop, I.M. The effect of sustainable feeding systems, combining total mixed rations and pasture, on milk fatty acid composition and antioxidant capacity in Jersey dairy cows. *Animals*. **2022**, *12*, 908. <https://doi.org/10.3390/ani12070908>
128. White, S.L.; Bertrand, J.A.; Wade, M.R.; Washburn, S.P.; Green Jr, J.T.; Jenkins, T.C. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. *J. Dairy Sci.* **2001**, *84*, 2295-2301. [https://doi.org/10.3168/jds.S0022-0302\(01\)74676-0](https://doi.org/10.3168/jds.S0022-0302(01)74676-0)
129. O’Callaghan, T.F.; Faulkner, H.; McAuliffe, S.; O’Sullivan, M.G.; Hennessy, D.; Dillon, P.; Kilcawley, K.N.; Stanton, C.; Ross, R.P. Quality characteristics, chemical composition, and sensory properties of butter from cows on pasture versus indoor feeding systems. *J. Dairy Sci.* **2016**, *99*, 9441-9460. <https://doi.org/10.3168/jds.2016-11271>
130. Barca, J.O.; Carriquiry, M.A.; Olazabal, L.A.; Fajardo, M.A.; Chilibroste, P.A.; Meikle, A. Milk fatty acid profile from cows fed with mixed rations and different access time to pastureland during early lactation. *J Anim Physiol Anim Nutr.* **2018**, *102*, 620-629. <https://doi.org/10.1111/jpn.12826>
131. Mangwe, M.; Bryant, R.; Gregorini, P. Rumen fermentation and fatty acid composition of milk of mid lactating dairy cows grazing chicory and ryegrass. *Animals*. **2020**, *10*, 169. <https://doi.org/10.3390/ani10010169>

132. Muir, S.K.; Ward, G.N.; Jacobs, J.L. Herbage intake and milk production of late-lactation dairy cows offered a second-year chicory crop during summer. *J. Dairy Sci.* **2015**, *98*, 8825-8835. <https://doi.org/10.3168/jds.2014-9147>
133. Kliem, K.E.; Morgan, R.; Humphries, D.J.; Shingfield, K.J.; Givens, D.I. Effect of replacing grass silage with maize silage in the diet on bovine milk fatty acid composition. *Animal.* **2008**, *2*, 1850-1858. <https://doi.org/10.1017/S1751731108003078>
134. Hernández-Ortega, M.; Martínez-Fernández, A.; Soldado, A.; González, A.; Arriaga-Jordán, C.M.; Argamentoría, A.; de la Roza-Delgado, B.; Vicente, F. Effect of total mixed ration composition and daily grazing pattern on milk production, composition and fatty acids profile of dairy cows. *J. Dairy Res.* **2014**, *81*, 471-478. <https://doi.org/10.1017/S0022029914000399>
135. Kalač, P.; Samková, E. The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. *Czech J. Anim. Sci.* **2010**, *55*, 521-537. <https://doi.org/10.17221/2485-CJAS>
136. Hanuš, O.; Samková, E.; Křížová, L.; Hasoňová, L.; Kala, R. Role of fatty acids in milk fat and the influence of selected factors on their variability—A review. *Molecules*, **2018**, *23*, 1636. <https://doi.org/10.3390/molecules23071636>
137. Mierlita, D.; Santa, A.; Mierlita, S.; Daraban, S.V.; Suteu, M.; Pop, I.M.; Mintas, O.S.; Macri, A.M. The Effects of Feeding Milled Rapeseed Seeds with Different Forage: Concentrate Ratios in Jersey Dairy Cows on Milk Production, Milk Fatty Acid Composition, and Milk Antioxidant Capacity. *Life.* **2022**, *13*, 46. <https://doi.org/10.3390/life13010046>
138. Kliem, K.E.; Humphries, D.J.; Reynolds, C.K.; Morgan, R.; Givens, D.I. Effect of oilseed type on milk fatty acid composition of individual cows, and also bulk tank milk fatty acid composition from commercial farms. *Animal.* **2016**, *11*, 354-364. <https://doi.org/10.1017/S1751731116001403>
139. Mitsiopolou, C.; Sotirakoglou, K.; Labrou, N.E.; Tsiplakou, E. The effect of whole sesame seeds on milk chemical composition, fatty acid profile and antioxidant status in goats. *Livest. Sci.* **2021**, *245*, 104452. <https://doi.org/10.1016/j.livsci.2021.104452>
140. Delamaire, E.; Guinard-Flament, J. Longer milking intervals alter mammary epithelial permeability and the udder's ability to extract nutrients. *J. Dairy Sci.* **2006**, *89*, 2007-2016. [https://doi.org/10.3168/jds.S0022-0302\(06\)72268-8](https://doi.org/10.3168/jds.S0022-0302(06)72268-8)

141. Wilde, C.J.; Knight, C.H. Milk yield and mammary function in goats during and after once-daily milking. *J. Dairy Res.* **1990**, *57*, 441–447. <https://doi.org/10.1017/S0022029900029484>
142. Farr, V.C.; Stelwagen, K.; Kerr, M.A.; Davis, S.R.; Eichler S.J. Effect of once daily milking (ODM) on enzyme activities in the bovine mammary gland. *Proc. N. Z. Soc. Anim. Prod.* **1995**, *55*, 12–13.
143. Goddard, M.E.; Hayes, B.J. Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nat. Rev. Genet.* **2009**, *10*, 381–391. <https://doi.org/10.1038/nrg2575>
144. Lung, L.H.; Petrini, J.; Ramírez-Díaz, J.; Salvian, M.; Rovadoscki, G.A.; Pilonetto, F.; Dauria, B.D.; Machado, P.F.; Coutinho, L.L.; Wiggans, G.R.; Mourão, G.B. Genome-wide association study for milk production traits in a Brazilian Holstein population. *J. Dairy Sci.* **2019**, *102*, 5305–5314. <https://doi.org/10.3168/jds.2018-14811>
145. Atashi, H.; Wilmot, H.; Vanderick, S.; Hubin, X.; Gengler, N. Genome-wide association study for milk production traits in Dual-Purpose Belgian Blue cows. *Livest. Sci.* **2022**, *256*, 104831. <https://doi.org/10.1016/j.livsci.2022.104831>
146. Jayawardana, J.M.D.R.; Lopez-Villalobos, N.; McNaughton, L.R.; Hickson, R.E. Genomic Regions Associated with Milk Composition and Fertility Traits in Spring-calved Dairy Cows in New Zealand. *Genes*, **2023**, *14*, 860. <https://doi.org/10.3390/genes14040860>
147. Dadousis, C.; Biffani, S.; Cipolat-Gotet, C.; Nicolazzi, E.L.; Rossoni, A.; Santus, E.; Bittante, G.; Cecchinato, A. Genome-wide association of coagulation properties, curd firmness modeling, protein percentage, and acidity in milk from Brown Swiss cows. *J. Dairy Sci.* **2016**, *99*, 3654–3666. <https://doi.org/10.3168/jds.2015-10078>
148. Tyrisevä, A.M.; Elo, K.; Kuusipuro, A.; Vilva, V.; Janonen, I.; Karjalainen, H.; Ikonen, T.; Ojala, M. Chromosomal regions underlying noncoagulation of milk in Finnish Ayrshire cows. *Genetics*. **2008**, *180*, 1211–1220. <https://doi.org/10.1534/genetics.107.083964>
149. Sanchez, M.P.; Ramayo-Caldas, Y.; Wolf, V.; Laithier, C.; El Jabri, M.; Michenet, A.; Boussaha, M.; Taussat, S.; Fritz, S.; Delacroix-Buchet, A.; Brochard, M. Sequence-based GWAS, network and pathway analyses reveal genes co-associated with milk cheese-making properties and milk composition in

- Montbéliarde cows. *Genet. Sel.* **2019**, *51*, 1-9. <https://doi.org/10.1186/s12711-019-0473-7>
150. Dadousis, C.; Pegolo, S.; Rosa, G.J.; Bittante, G.; Cecchinato, A. Genome-wide association and pathway-based analysis using latent variables related to milk protein composition and cheesemaking traits in dairy cattle. *J. Dairy Sci.* **2017**, *100*, 9085-9102. <https://doi.org/10.3168/jds.2017-13219>
151. Marina, H.; Pelayo, R.; Suárez-Vega, A.; Gutiérrez-Gil, B.; Esteban-Blanco, C.; Arranz, J.J. Genome-wide association studies (GWAS) and post-GWAS analyses for technological traits in Assaf and Churra dairy breeds. *J. Dairy Sci.* **2021**, *104*, 11850-11866. <https://doi.org/10.3168/jds.2021-20510>
152. Lopez-Villalobos, N.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Sneddon, N.W.; Holroyd, S.E.; MacGibbon, A.K.; Snell, R.G. Genetic correlations of milk fatty acid contents predicted from milk mid-infrared spectra in New Zealand dairy cattle. *J. Dairy Sci.* **2020**, *103*, 7238-7248. <https://doi.org/10.3168/jds.2019-17971>
153. Tumino, S.; Criscione, A.; Moltisanti, V.; Marletta, D.; Bordonaro, S.; Avondo, M.; Valenti, B. Feeding system resizes the effects of dgat1 polymorphism on milk traits and fatty acids composition in Modicana cows. *Animals.* **2021**, *11*, 1616. <https://doi.org/10.3390/ani11061616>
154. Li, Y.; Zhou, H.; Cheng, L.; Edwards, G.R.; Hickford, J.G. Effect of DGAT1 variant (K232A) on milk traits and milk fat composition in outdoor pasture-grazed dairy cattle. *N. Z. J. Agric. Res.* **2021**, *64*, 101-13. <https://doi.org/10.1080/00288233.2019.1589537>
155. Stoop, W.M.; Schennink, A.; Visker, M.H.P.W.; Mullaart, E.; Van Arendonk, J.A.M.; Bovenhuis, H. Genome-wide scan for bovine milk-fat composition. I. Quantitative trait loci for short-and medium-chain fatty acids. *J. Dairy Sci.* **2009**, *92*, 4664-4675. <https://doi.org/10.3168/jds.2008-1966>
156. Schennink, A.; Bovenhuis, H.; Léon-Kloosterziel, K.M.; Van Arendonk, J.A.; Visker, M.H. Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. *Anim. Genet.* **2009**, *40*, 909-916. <https://doi.org/10.1111/j.1365-2052.2009.01940.x>
157. Olsen, H.G.; Knutsen, T.M.; Kohler, A.; Svendsen, M.; Gidskehaug, L.; Grove, H.; Nome, T.; Sodeland, M.; Sundsaasen, K.K.; Kent, M.P.; Martens, H. Genome-wide association mapping for milk fat composition and fine mapping of a QTL

- for *de novo* synthesis of milk fatty acids on bovine chromosome 13. *Genet. Sel.* **2017**, *49*, 1-3. <https://doi.org/10.1186/s12711-017-0294-5>
158. Knutsen, T.M.; Olsen, H.G.; Ketto, I.A.; Sundsaasen, K.K.; Kohler, A.; Tafintseva, V.; Svendsen, M.; Kent, M.P.; Lien, S. Genetic variants associated with two major bovine milk fatty acids offer opportunities to breed for altered milk fat composition. *Genet. Sel.* **2022**, *54*, 35. <https://doi.org/10.1186/s12711-022-00731-9>
159. Palombo, V.; Milanese, M.; Sgorlon, S.; Capomaccio, S.; Mele, M.; Nicolazzi, E.; Ajmone-Marsan, P.; Pilla, F.; Stefanon, B.; D'Andrea, M. Genome-wide association study of milk fatty acid composition in Italian Simmental and Italian Holstein cows using single nucleotide polymorphism arrays. *J. Dairy Sci.* **2018**, *101*, 11004-11019. <https://doi.org/10.3168/jds.2018-14413>
160. Tiplady, K.M.; Lopdell, T.J.; Sherlock, R.G.; Johnson, T.J.; Spelman, R.J.; Harris, B.L.; Davis, S.R.; Littlejohn, M.D.; Garrick, D.J. Comparison of the genetic characteristics of directly measured and Fourier-transform mid-infrared-predicted bovine milk fatty acids and proteins. *J. Dairy Sci.* **2022**, *105*, 9763-9791. <https://doi.org/10.3168/jds.2022-22089>

### **Foreword to Chapter 3**

No. 4 Dairy farm (twice-a-day milking) consisted of approximately 560 cows and in the 2020-2021 production season the herd was divided into 3 mobs for experiment purposes. For this experiment, the young mob containing first and second parity Holstein-Friesian and Holstein-Friesian × Jersey cows was selected. The cows in the young mob were fed more pasture compared to other two mobs. To make a comparable match, first and second parity Holstein-Friesian and Holstein-Friesian × Jersey cows from the No. 1 Dairy farm (once-a-day milking) were selected for this chapter. The majority of the diet of cows on both farms was pasture. The chemical composition of the diet of cows on both farms was similar.



### Chapter 3

#### **Fatty acid composition of dairy milk: A case study comparing once- and twice-a-day milking of pasture-fed cows at different stages of lactation**

Published as:

Sanjayanj, I.; Lopez-Villalobos, N.; Blair, H.T.; Janssen, P.W.M.; Holroyd, S.E.; MacGibbon, A.K.H. Fatty acid composition of dairy Milk: A case study comparing once- and twice-a-day milking of pasture-fed cows at different stages of lactation. *Dairy*. **2022**, *3*, 174–189. <https://doi.org/10.3390/dairy3010014>



### 3.1. Abstract

In this case study, we compared the gross composition and fatty acid composition of milk from cows milked once a day (OAD) and twice a day (TAD) at different stages of lactation in real farm conditions with no control on feed. Seventy-two cows from a OAD milking herd and 181 cows from a TAD milking herd were sampled in early, mid- and late lactation. Calibration equations were developed to enable the prediction of proportions of individual fatty acids using mid-infrared spectroscopy. Cows milked OAD produced 25% lower daily milk yield compared with cows milked TAD. Percentages of fat and protein were 21% and 9% higher in cows milked OAD compared with cows milked TAD, respectively. The proportion of saturated fatty acids (SFA) was significantly lower, while the proportions of *de novo* synthesised fatty acid from C8:0 to C14:0 were significantly higher, in cows milked OAD compared with cows milked TAD. Once-a-day milking improved the energy balance of cows, which led to higher proportions of *de novo* synthesised fatty acids and lower proportions of long-chain fatty acids (16:0 and above). The proportion of SFA was significantly higher in mid-lactation compared with early lactation and late lactation in cows milked OAD and TAD. In early lactation, the proportions of C4:0 to C12:0 fatty acids in cows milked OAD were significantly higher compared with cows milked TAD due to the improved energy status of cows milked OAD. Understanding the proportions of individual fatty acids in cows milked OAD and TAD will enable further studies on milk fat characteristics, butter hardness, and coagulation properties of milk.

Keywords: fatty acid; milk composition; milking frequency; stage of lactation; dairy cow; New Zealand

### 3.2. Introduction

Milk composition affects the yield and quality of dairy products [1]. Fat and protein are major components in milk that influence the final yield and quality of dairy products, especially cheese [2,3] and butter [4]. Milk fat is mainly composed of fatty acids, with cow milk comprising 70% saturated fatty acids (SFA) and 30% unsaturated fatty acids (UFA) [5]. The proportions of different fatty acids in milk affects the quality of milk and dairy products. Fatty acids in the milk, such as C8:0, C10:0, C12:0, C18:1 and C18:2, affect the depth of curd formed during cheese making [2]. The variation in milk composition and fatty acid composition is caused by several factors, including genetics,

breed, feed, season, stage of lactation, and age of cows [3,6,7]. Proportions of UFA were lower in mid-lactation compared with early and late lactation [8–10]. Milk from late lactation cows could have different processing properties compared with milk from early and mid-lactation cows [11].

In New Zealand, dairy farmers are paid for the quantity of milk fat and protein with a penalty for milk volume. Traditionally, cows are milked twice a day (TAD). Over the last two decades, some farmers have adopted once-a-day (OAD) milking for the whole season or part of the season due to several benefits for the farmer and the cow [12]. Once-a-day milking reduces feed, labour, and management costs, improves labour and farm efficiency, and provides farmers with an alternative employment option [13,14]. The reproductive performance of cows milked OAD is significantly improved compared with cows milked TAD [15,16]. However, OAD milking reduces the yields of milk, fat, and protein which are the main concerns for farmers interested in practicing OAD milking [14]. Once-a-day milking could modify the composition of milk as milk synthesis and milk secretion are likely to be reduced by OAD milking in dairy cows [17]. Fat and protein percentages increased, and lactose percentage decreased during OAD milking in both short [13] and full [15,16] lactation studies. The changes in the milk composition could influence the processing characteristics of the milk. Once-a-day milking tends to affect cheese coagulation properties by increasing rennet clotting time and increasing curd firming time [18] or improving curd firmness [19], although one study showed no change in these properties [20].

There have been no studies exploring the effect of milking frequency on milk fat composition. Therefore, the objective of this case study was to compare the gross composition and fatty acid composition of milk from cows milked OAD and TAD at different stages of lactation in real farm conditions with no control on feed.

### **3.3. Materials and Methods**

#### **3.3.1. Farm data**

This study was conducted at Massey University, Palmerston North dairy farms from July 2020 to March 2021. The farms are located in the lower North Island of New Zealand (longitude 175°, latitude -40°), adjacent to each other, and therefore cows experienced the same weather conditions but different management systems for feeding and milk harvesting.

The Massey University No. 1 Dairy farm is managed as a low-input system, with cows milked OAD throughout the season. The stocking rate of the farm is 2.4 cows/ha, with cows mainly grazing on fresh ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture, with lower level of supplements and periodic allocation of grazing crops. Cows were milked daily at 6.30 am. After milking each morning, all cows were allocated a fresh strip of pasture or crops as a single herd.

The Massey University No. 4 Dairy farm is managed as a high-input system, with cows milked TAD over the entire lactation period. The stocking rate is 2.6 cows/ha. Ryegrass-white clover pasture is given as a main feed source, but with a higher level of supplementation than the No. 1 Dairy farm. Cows were milked daily at 5.30 am and 2.30 pm. After milking each morning and evening, all cows were allocated a fresh strip of pasture or crop as a single herd. The approximate values of diet composition and chemical composition of feed given to cows in both farms during the study period are shown in Table 3.1.

In early lactation, cows at the No.1 Dairy farm were fed approximately 8 kg DM/cow/day pasture, 4 kg DM/cow/day herb mix crop containing chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and red clover (*Trifolium pratense*), and lower supplements (maize silage, tapioca, and dried distillers grains). Cows at the No. 4 Dairy farm were fed approximately 17 kg DM/cow/day pasture, with maize silage, concentrate (soy meal), and dry roughage. In mid-lactation, No. 1 Dairy cows were fed approximately 12 kg DM/cow/day pasture and 4 kg DM/cow/day crop mix without any supplements whereas No. 4 Dairy cows were fed approximately 17 kg DM/cow/day pasture with higher supplements (maize silage, dried distillers grains, and grass baleage). In the late lactation, cows at No.1 Dairy farm were fed approximately 4.5 kg DM/cow/day pasture, 3 kg DM/cow/day herb mix crop, with tapioca pellets, concentrate (corn gluten feed pellets), and Lucerne baleage. Cows at the No. 4 Dairy farm were fed approximately 10 kg DM/cow/day pasture, with maize silage, concentrate (corn gluten feed pellets), straw, and grass baleage. Feed composition for cows at No. 1 Dairy farm and No. 4 Dairy farm in early, mid-, and late lactation are presented in Tables 1 and 2 of Appendix 1.

The feed sampling was carried out 24 h before each milk sampling using the same method described by Correa-Luna et al. [21]. The samples were freeze-dried, ground, and analyzed with the near-infrared reflectance spectrometry technique for the chemical

composition of feed [22]. No specific software was used for feed balancing. The level of feeding and feed composition in each farm was defined by the farm manager, who feeds the cows as a group attempting to satisfy the requirements for metabolizable energy for maintenance, live weight gain, activity, and milk production.

The limitation of this study was the confounding effect of feed and milking frequency, there was no control practiced in this study. The feed provided during the experimental period was not identical. The cows milked TAD were fed slightly higher supplements than cows milked OAD.

**Table 3.1.** Dietary and chemical composition of feed offered at the No. 1 and No. 4 Dairy farms during the sampling periods (early, mid-, and late lactation) in the 2020–2021 production season.

Lactation stage	No. 1 Dairy farm (OAD <sup>12</sup> )			No. 4 Dairy farm (TAD <sup>13</sup> )		
	Early (September)	Mid (December)	Late (March)	Early (September)	Mid (December)	Late (March)
Diet composition						
(kg DM <sup>1</sup> per cow per day)						
Pasture	8.0	12.0	4.5	17.0	17.0	10.0
Herb mix crop <sup>2</sup>	4.0	4.0	3.0	-	-	-
Maize silage	1.0	-	-	5.0	5.0	2.0
DDG <sup>3</sup>	1.5	-	-	-	1.0	-
Tapioca pellets	1.5	-	1.5	-	-	-
Concentrate <sup>4</sup>	-	-	2.0	4.0	-	1.0
Dry roughage <sup>5</sup>	-	-	-	0.2	-	1.0
Baleage <sup>6</sup>	-	-	4.0	-	1.0	10
Diet chemical composition						
ME <sup>7</sup> MJ/kg DM	11.89	12.12	9.98	11.36	10.58	11.28
CP <sup>8</sup> g/100 g DM	20.08	19.48	21.91	19.49	19.08	21.27
NDF <sup>9</sup> g/100 g DM	38.18	38.66	39.88	44.13	43.48	45.69
ADF <sup>10</sup> g/100 g DM	20.94	20.38	26.00	20.71	23.60	25.40
SSS <sup>11</sup> g/100 g DM	16.77	14.11	8.79	19.13	17.55	8.59
Lipid g/100 g DM	4.08	4.69	4.54	4.40	4.09	4.77

<sup>1</sup> DM = dry matter; <sup>2</sup> Comprises chicory (*Cichorium intybus*), red clover (*Trifolium pratense*), and plantain (*Plantago lanceolata*); <sup>3</sup> dried distillers grains; <sup>4</sup> grain-based concentrate; <sup>5</sup> hay and straw; <sup>6</sup> Lucerne baleage in the No. 1 Dairy farm and grass baleage in the No. 4 Dairy farm; <sup>7</sup> ME = metabolisable energy; <sup>8</sup> CP = crude protein; <sup>9</sup> NDF = neutral detergent fibre; <sup>10</sup> ADF = acid detergent fibre; <sup>11</sup> SSS = soluble sugars and starch; <sup>12</sup> OAD = once-a-day milking; <sup>13</sup> TAD = twice-a-day milking; baleage is partly dried forage, with 45–55% moisture and is baled and wrapped with at least 6 layers of 0.025 mm plastic.

### ***3.3.2. Sample collection***

Milk sample collection was conducted on both farms during the 2020–2021 production season during early (September,  $49 \pm 15$  days in milk), mid- (December,  $129 \pm 12$  days in milk) and late (March,  $229 \pm 13$  days in milk) lactation. Composite milk samples were collected using Waikato milk flow meters from 72 No. 1 Dairy farm cows and 181 No. 4 Dairy farm cows. The cows from the No. 1 Dairy farm consisted of 39 Holstein-Friesian (F) and 33 Holstein-Friesian  $\times$  Jersey (F $\times$ J) cows. The cows from the No. 4 Dairy farm herd consisted of 101 F and 80 F $\times$ J cows. All the cows sampled from the two farms were 1<sup>st</sup> and 2<sup>nd</sup> lactation cows. Milk samples were refrigerated at 0–4°C until analysis, which was carried out within two days of each sampling in September, December, and March.

### ***3.3.3. Milk composition and fatty acid analysis***

Gross composition and individual fatty acids were determined using Milkoscan FT1 (Foss Analytical, Hillerød, Denmark). A total of 115 milk samples were collected during the 2020–2021 production season from 1<sup>st</sup> and 2<sup>nd</sup> lactation F and F $\times$ J cows during early lactation (mid-September). One part of the 115 individual milk samples was analysed for proportions of fatty acids by fatty acid methyl ester analysis using gas chromatography (GC) [23] with Shimadzu GC-2010 plus. The remaining part of the milk samples was analysed for fatty acids in Milkoscan FT1 (Foss Analytical, Hillerød, Denmark). Calibration equations for individual fatty acids were developed using FTIR calibrator software (Foss Analytical, Hillerød, Denmark). The calibration equations were created using 95 samples for training and 20 samples for validation. The coefficient of determination ( $R^2$ ) values for all fatty acid equations were more than 0.8. The developed calibration equations were used to predict the proportions of individual fatty acids of milk collected from 253 cows in early, mid-, and late lactation using the Milkoscan FT1 machine. Percentages of fat, protein and lactose were analysed using standard calibrations from Foss. The milk composition of samples collected evening and morning in TAD milking (No. 4 Dairy farm) was analysed separately, and a weighted (by milk yield) average was used in analyses.

### ***3.3.4. Body condition score***

Body condition score (BCS) was measured during the 2020–2021 production season by a single research technician using a 10-point scale [24]. The BCS of each cow in each milking frequency was recorded every month from calving to the dry-off date.

### 3.3.5. Statistical analysis

Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Least-square means and standard errors of the variables were obtained using the following linear mixed model using the MIXED procedure. In this study, the feed was a confounding variable, and was not included in the model.

$$Y_{ijklm} = \mu + M_i + B_j(M_i) + L_k + S_l + MS_{il} + \beta_1 dm + \beta_2 dm^2 + C_m + e_{ijklm}$$

$Y_{ijklm}$  is the observation for milk yield traits and composition traits for milking frequency  $i$ , breed group  $j$ , lactation number  $k$ , stage of lactation  $l$  and cow  $m$ .

$\mu$  is the population mean.

$M_i$  is the fixed effect of milking frequency  $i$  ( $i = \text{OAD and TAD}$ ).

$B_j(M_i)$  is the fixed effect of breed  $j$  nested in milking frequency  $i$  ( $j = \text{F and F} \times \text{J}$ ).

$L_k$  is the fixed effect of lactation number ( $k = 1^{\text{st}}$  and  $2^{\text{nd}}$  lactation).

$S_l$  is the fixed effect of stage of lactation  $k$  ( $l = \text{early, mid and late}$ ).

$MS_{il}$  is the fixed effect of interaction between milking frequency  $i$  and stage of lactation  $l$ .

$\beta_1$  is the regression coefficient associated with the linear effect of deviation from median calving date of cow  $m$ .

$\beta_2$  is the regression coefficient associated with the quadratic effect of deviation from median calving date of cow  $m$ .

$C_m$  is the random effect of cow  $m$  ( $m = 1, 2, \dots, 253$ ) assumed with mean zero and variance  $\sigma_c^2$ .

$e_{ijklm}$  is the residual random error assumed with mean zero and variance  $\sigma_e^2$ .

Least-square means and standard errors for milking frequency and combinations of milking frequency and stages of lactation were obtained and used for multiple mean comparisons using Fisher's least significant difference  $t_{ij} = (\bar{y}_i - \bar{y}_j) / \hat{\sigma}_{ij}$ , where  $i$  and  $j$  are subscripts for two least squares either for each milking frequency or combination of milking frequency and stage of lactation, and  $\hat{\sigma}_{ij}$  is the square root of the estimated variance of  $\bar{y}_i - \bar{y}_j$  calculated as  $\hat{\sigma}_{ij}^2 = s^2(1/w_i + 1/w_j)$ , where  $w_i$  and  $w_j$  are the sums of the weights in groups  $i$  and  $j$ . If  $|\bar{y}_i - \bar{y}_j| \geq t(\alpha; v) \sqrt{\hat{\sigma}_{ij}^2}$ , then the least-square means were declared significantly different. In this last equation, the right-hand side is Fisher's least significant difference, with  $t(\alpha; v)$  being the two-tailed critical value from Student's

t distribution with a significant level  $\alpha$  and with a  $\nu$  degree of freedom of the mean square of the error. Significant differences were declared at  $p < 0.05$ .

Monthly records of BCS, from calving to dry off, of each cow in each milking frequency were used to model BCS curves with a third-order polynomial.

### 3.4. Results

Descriptive statistics of daily yields of milk components, gross milk composition, and fatty acid composition of all the samples (regardless of milking frequency and stage of lactation) analysed are presented in Table 3.2.

**Table 3.2.** Descriptive statistics of daily yields of milk, fat, protein and lactose, gross milk composition, and fatty acid composition of cows milked during the 2020–2021 production season.

Variable	N	Mean	SD	CV%	Min	Max
Milk yield, kg/day	755	18.69	5.73	31.6	4.74	40.66
Fat yield, kg/day	755	0.85	0.28	32.4	0.26	5.22
Protein yield, kg/day	755	0.68	0.17	25.4	0.21	1.54
Lactose yield, kg/day	755	0.91	0.31	34.3	0.19	2.08
Fat %	759	4.27	0.96	22.6	1.27	8.53
Protein %	759	3.87	0.41	10.7	2.74	5.67
Lactose %	759	4.97	0.26	5.2	4.07	5.51
Fatty acid, % of the total FA						
SFA <sup>1</sup>	759	69.94	3.04	4.3	57.10	77.94
UFA <sup>2</sup>	759	30.23	2.60	8.6	23.70	41.44
PUFA <sup>3</sup>	759	2.94	0.45	15.2	1.49	4.40
C4:0	759	4.03	0.30	7.5	2.50	4.65
C6:0	759	2.81	0.22	7.8	1.37	3.31
C8:0	759	1.51	0.15	9.7	0.56	1.91
C10:0	759	3.40	0.42	12.2	0.93	4.83
C12:0	759	3.80	0.35	9.3	2.17	5.06
C14:0	759	12.68	1.24	9.8	7.14	15.33
C16:0	759	31.89	2.46	7.7	24.40	39.81
C18:0	759	13.04	1.42	10.9	7.87	22.23
C18:1 <i>cis</i> -9	759	20.31	2.70	13.3	14.75	32.52
Omega6	759	1.59	0.36	22.8	0.37	2.77
SCFA <sup>4</sup>	759	8.35	0.61	7.4	4.43	9.71
MCFA <sup>5</sup>	759	19.89	1.76	8.8	11.46	24.41
LCFA <sup>6</sup>	759	66.83	2.57	3.9	59.12	82.02

<sup>1</sup> SFA = saturated fatty acids; <sup>2</sup> UFA = unsaturated fatty acid; <sup>3</sup> PUFA = polyunsaturated fatty acids; <sup>4</sup> SCFA = short-chain fatty acids (sum of C4:0, C6:0 and C8:0); <sup>5</sup> MCFA = medium-chain fatty acids (sum of C10:0, C12:0 and C14:0); <sup>6</sup> LCFA = long-chain fatty acids (sum of C16:0, C18:0, C18:1 *cis*-9 and Omega6).

Least-square means and standard errors for the study variables for both milking frequencies are presented in Table 3.3. The least-square means were calculated for the entire season and the weighted average by milk yield was used to calculate the milk composition of samples from cows milked TAD. Cows milked OAD produced 25% less daily milk yield, 13% less fat yield, 16% less protein yield, and 28% less lactose yield compared with cows milked TAD.

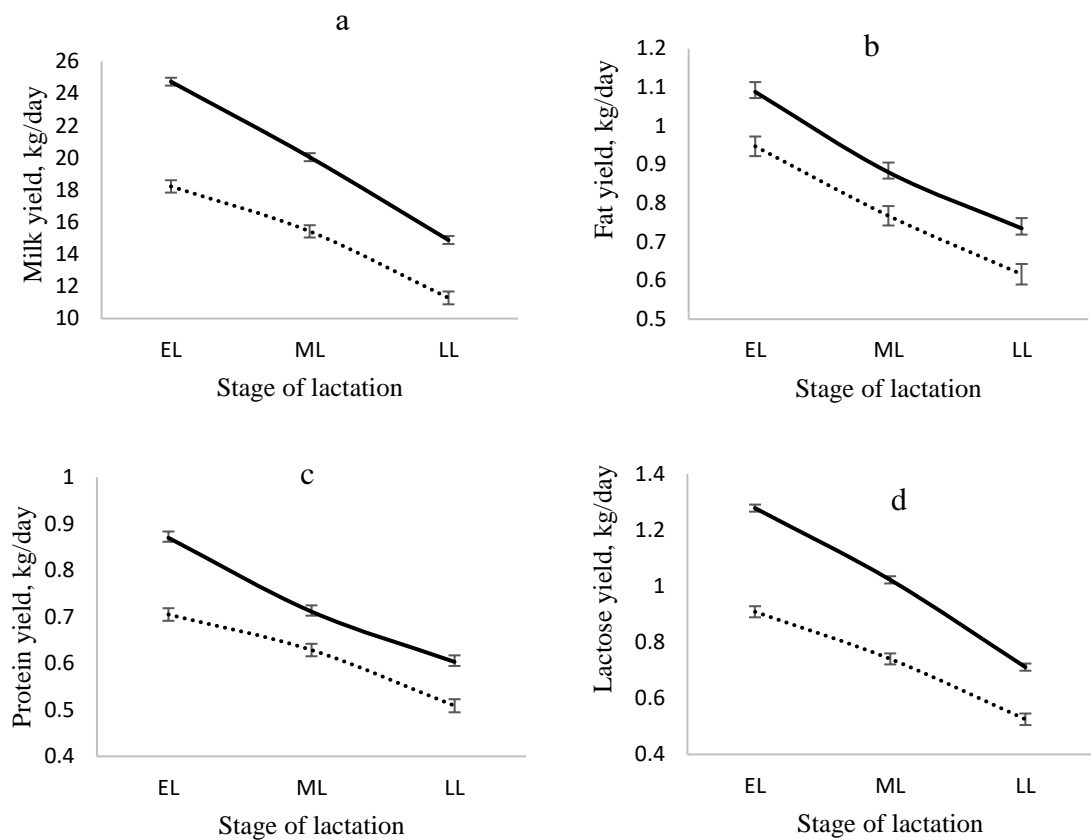
**Table 3.3.** Least-square means and standard errors of daily yields of milk, fat, protein, and lactose, gross milk composition, and fatty acid composition of cows milked once a day (OAD) and twice a day (TAD) during the 2020–2021 production season.

Variable	Milking Frequency		p-Value
	OAD	TAD	
Milk yield, kg/day	15.43 ± 0.3	20.49 ± 0.19	<0.001
Fat yield, kg/day	0.78 ± 0.02	0.90 ± 0.01	<0.001
Protein yield, kg/day	0.61 ± 0.01	0.73 ± 0.01	<0.001
Lactose yield, kg/day	0.72 ± 0.02	1.00 ± 0.01	<0.001
Fat %	5.03 ± 0.08	3.99 ± 0.05	<0.001
Protein %	4.12 ± 0.03	3.76 ± 0.02	<0.001
Lactose %	4.80 ± 0.02	5.02 ± 0.01	<0.001
Fatty acid, % of the total FA			
SFA <sup>1</sup>	69.47 ± 0.21	70.47 ± 0.13	<0.001
UFA <sup>2</sup>	31.03 ± 0.17	29.59 ± 0.11	<0.001
PUFA <sup>3</sup>	3.11 ± 0.02	2.83 ± 0.02	<0.001
C4:0	3.83 ± 0.02	4.12 ± 0.01	<0.001
C6:0	2.76 ± 0.01	2.84 ± 0.01	<0.001
C8:0	1.53 ± 0.01	1.51 ± 0.01	0.0190
C10:0	3.57 ± 0.03	3.36 ± 0.02	<0.001
C12:0	4.00 ± 0.02	3.74 ± 0.02	<0.001
C14:0	12.83 ± 0.08	12.72 ± 0.05	0.2493
C16:0	31.31 ± 0.18	32.39 ± 0.12	<0.001
C18:0	12.62 ± 0.11	13.24 ± 0.07	<0.001
C18:1 <i>cis</i> -9	19.9 ± 0.17	20.21 ± 0.11	0.1310
Omega6	1.67 ± 0.02	1.53 ± 0.01	<0.001
SCFA <sup>4</sup>	8.12 ± 0.04	8.46 ± 0.03	<0.001
MCFA <sup>5</sup>	20.4 ± 0.13	19.82 ± 0.08	0.0002
LCFA <sup>6</sup>	65.5 ± 0.19	67.37 ± 0.12	<0.001

<sup>1</sup> SFA = saturated fatty acids; <sup>2</sup> UFA = unsaturated fatty acid; <sup>3</sup> PUFA = polyunsaturated fatty acid; <sup>4</sup> SCFA = short-chain fatty acids (sum of C4:0, C6:0 and C8:0); <sup>5</sup> MCFA = medium-chain fatty acids (sum of C10:0, C12:0 and C14:0); <sup>6</sup> LCFA = long-chain fatty acids (sum of C16:0, C18:0, C18:1 *cis*-9 and Omega6).

The percentage of fat was 21% higher, and the percentage of protein was 9% higher in cows milked OAD compared with cows milked TAD. The percentage of lactose was

significantly lower in cows milked OAD compared with cows milked TAD. The proportion of SFA was lower in cows milked OAD compared with cows milked TAD, while the proportion of UFA in cows milked OAD was higher than in cows milked TAD. The proportion of PUFA was higher in cows milked OAD than in cows milked TAD. The proportions of short-chain fatty acids (SCFA) and long-chain fatty acids (LCFA) were significantly lower, and the proportion of medium-chain fatty acids (MCFA) was significantly higher in cows milked OAD.

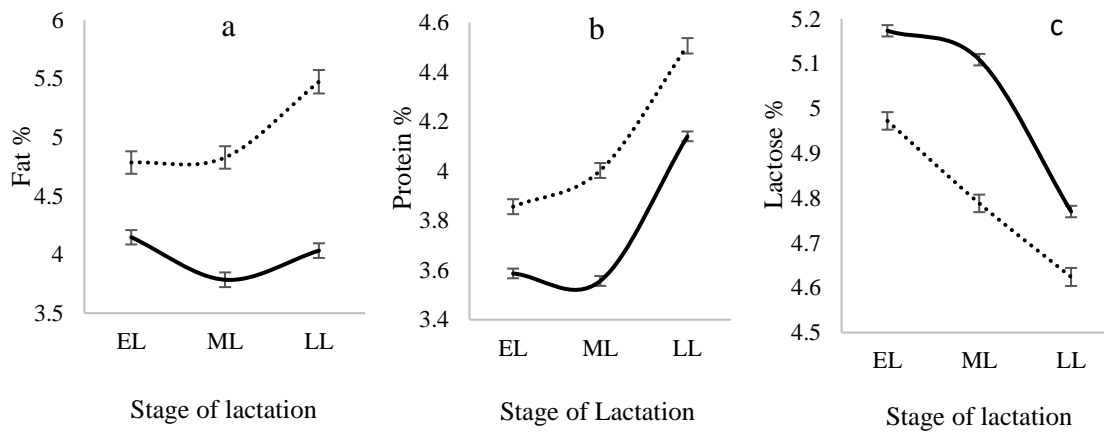


**Figure 3.1.** Daily yields of (a) milk, (b) fat, (c) protein, and (d) lactose in cows milked OAD (....) and TAD (—) during the 2020–2021 production season.

EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show the standard errors. The p-values for the interaction between milking frequency and stage of lactation were <0.0001 for daily yield of milk, fat, protein, and lactose.

Daily yields of milk, fat, protein, and lactose decreased from early to late lactation (Figure 3.1) with TAD milking producing higher yields than OAD milking at all stages of lactation. Fat and protein percentages were higher ( $p < 0.05$ ) in cows milked OAD than

in cows milked in TAD, while lactose percentage was lower ( $p < 0.05$ ) in cows milked OAD than in cows milked TAD (Table 3.4 and Figure 3.2) throughout the season. Table 3.4 shows the least-square means and the standard errors for all the study variables for three lactation stages in both milking frequencies. Figures 3.1–3.4 show the pattern of daily yield traits, milk composition, and proportions of some groups of fatty acids throughout the stages of lactation for each milking frequency.



**Figure 3.2.** Percentage of (a) fat, (b) protein, and (c) lactose in cows milked OAD (.....) and TAD (—) during the 2020–2021 production season.

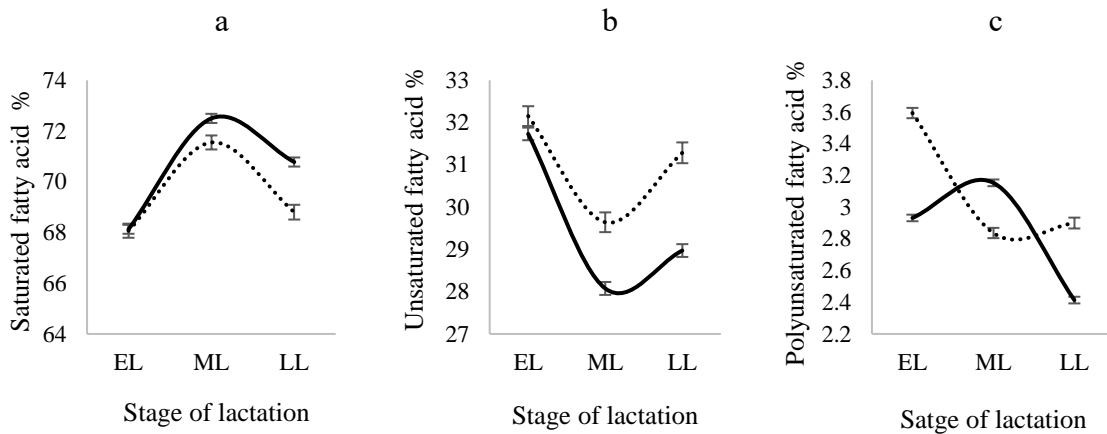
EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show the standard errors. The  $p$ -values for the interaction between milking frequency and stage of lactation were  $<0.0001$  for fat, protein, and lactose percentages.

**Table 3.4.** Least-square means and standard errors of daily yields of milk, fat, protein, and lactose, gross milk composition, and fatty acid composition of cows milked once a day (OAD) and twice a day (TAD) in early, mid-, and late lactation during the 2020–2021 production season.

Variable	OAD			TAD			p Value MF×SOL <sup>7</sup>
	Early	Mid	Late	Early	Mid	Late	
Milk yield, kg/day	18.77 ± 0.38 <sup>c</sup>	15.89 ± 0.38 <sup>d</sup>	11.63 ± 0.40 <sup>f</sup>	25.47 ± 0.24 <sup>a</sup>	20.64 ± 0.25 <sup>bd</sup>	15.34 ± 0.25 <sup>e</sup>	<0.001
Fat yield, kg/day	0.95 ± 0.03 <sup>b</sup>	0.77 ± 0.03 <sup>e</sup>	0.62 ± 0.03 <sup>e</sup>	1.09 ± 0.02 <sup>a</sup>	0.88 ± 0.02 <sup>c</sup>	0.74 ± 0.02 <sup>d</sup>	<0.001
Protein yield, kg/day	0.70 ± 0.01 <sup>b</sup>	0.63 ± 0.01 <sup>c</sup>	0.51 ± 0.01 <sup>d</sup>	0.87 ± 0.01 <sup>a</sup>	0.71 ± 0.01 <sup>b</sup>	0.60 ± 0.01 <sup>c</sup>	<0.001
Lactose yield, kg/day	0.91 ± 0.02 <sup>c</sup>	0.74 ± 0.02 <sup>d</sup>	0.53 ± 0.02 <sup>f</sup>	1.28 ± 0.01 <sup>a</sup>	1.02 ± 0.01 <sup>b</sup>	0.71 ± 0.01 <sup>de</sup>	<0.001
Fat %	4.78 ± 0.10 <sup>b</sup>	4.83 ± 0.10 <sup>b</sup>	5.47 ± 0.10 <sup>a</sup>	4.15 ± 0.06 <sup>c</sup>	3.78 ± 0.06 <sup>d</sup>	4.03 ± 0.06 <sup>c</sup>	<0.001
Protein %	3.86 ± 0.03 <sup>d</sup>	4.00 ± 0.03 <sup>c</sup>	4.51 ± 0.03 <sup>a</sup>	3.59 ± 0.02 <sup>e</sup>	3.56 ± 0.02 <sup>e</sup>	4.14 ± 0.02 <sup>b</sup>	<0.001
Lactose %	4.97 ± 0.02 <sup>c</sup>	4.79 ± 0.02 <sup>d</sup>	4.62 ± 0.02 <sup>e</sup>	5.17 ± 0.01 <sup>a</sup>	5.11 ± 0.01 <sup>b</sup>	4.77 ± 0.01 <sup>d</sup>	<0.001
Fatty acid, % of the total FA							
SFA <sup>1</sup>	68.07 ± 0.28 <sup>e</sup>	71.55 ± 0.28 <sup>b</sup>	68.8 ± 0.29 <sup>d</sup>	68.13 ± 0.18 <sup>de</sup>	72.5 ± 0.18 <sup>a</sup>	70.78 ± 0.18 <sup>c</sup>	<0.001
UFA <sup>2</sup>	32.15 ± 0.23 <sup>a</sup>	29.64 ± 0.23 <sup>c</sup>	31.29 ± 0.25 <sup>b</sup>	31.73 ± 0.15 <sup>ab</sup>	28.08 ± 0.15 <sup>e</sup>	28.97 ± 0.15 <sup>d</sup>	<0.001
PUFA <sup>3</sup>	3.59 ± 0.03 <sup>a</sup>	2.84 ± 0.03 <sup>c</sup>	2.90 ± 0.03 <sup>c</sup>	2.93 ± 0.02 <sup>c</sup>	3.15 ± 0.02 <sup>b</sup>	2.41 ± 0.02 <sup>d</sup>	<0.001
C4:0	4.05 ± 0.02 <sup>c</sup>	3.94 ± 0.02 <sup>d</sup>	3.49 ± 0.02 <sup>e</sup>	4.12 ± 0.02 <sup>b</sup>	4.31 ± 0.02 <sup>a</sup>	3.92 ± 0.02 <sup>d</sup>	<0.001
C6:0	2.93 ± 0.02 <sup>b</sup>	2.86 ± 0.02 <sup>c</sup>	2.49 ± 0.02 <sup>e</sup>	2.82 ± 0.01 <sup>c</sup>	2.99 ± 0.01 <sup>a</sup>	2.70 ± 0.01 <sup>d</sup>	<0.001
C8:0	1.69 ± 0.01 <sup>a</sup>	1.57 ± 0.01 <sup>b</sup>	1.34 ± 0.01 <sup>e</sup>	1.54 ± 0.01 <sup>c</sup>	1.57 ± 0.01 <sup>b</sup>	1.41 ± 0.01 <sup>d</sup>	<0.001
C10:0	3.94 ± 0.04 <sup>a</sup>	3.65 ± 0.04 <sup>b</sup>	3.11 ± 0.04 <sup>e</sup>	3.39 ± 0.02 <sup>d</sup>	3.55 ± 0.02 <sup>c</sup>	3.13 ± 0.02 <sup>e</sup>	<0.001
C12:0	4.15 ± 0.03 <sup>a</sup>	4.07 ± 0.03 <sup>a</sup>	3.78 ± 0.04 <sup>d</sup>	3.64 ± 0.02 <sup>e</sup>	3.91 ± 0.02 <sup>c</sup>	3.67 ± 0.02 <sup>e</sup>	<0.001
C14:0	12.04 ± 0.11 <sup>d</sup>	13.45 ± 0.11 <sup>a</sup>	13.0 ± 0.12 <sup>c</sup>	11.72 ± 0.07 <sup>e</sup>	13.03 ± 0.07 <sup>c</sup>	13.40 ± 0.07 <sup>a</sup>	<0.001
C16:0	29.83 ± 0.22 <sup>e</sup>	32.66 ± 0.22 <sup>c</sup>	31.43 ± 0.23 <sup>d</sup>	30.26 ± 0.14 <sup>e</sup>	33.70 ± 0.14 <sup>a</sup>	33.21 ± 0.14 <sup>b</sup>	<0.001
C18:0	11.92 ± 0.15 <sup>c</sup>	12.89 ± 0.15 <sup>b</sup>	13.05 ± 0.16 <sup>b</sup>	13.49 ± 0.10 <sup>a</sup>	13.43 ± 0.10 <sup>a</sup>	12.81 ± 0.1 <sup>b</sup>	<0.001
C18:1 <i>cis</i> -9	21.36 ± 0.24 <sup>b</sup>	18.18 ± 0.24 <sup>e</sup>	20.15 ± 0.25 <sup>c</sup>	22.62 ± 0.15 <sup>a</sup>	18.94 ± 0.16 <sup>d</sup>	19.08 ± 0.15 <sup>d</sup>	<0.001
Omega6	2.17 ± 0.03 <sup>a</sup>	1.53 ± 0.03 <sup>d</sup>	1.32 ± 0.03 <sup>e</sup>	1.63 ± 0.02 <sup>c</sup>	1.73 ± 0.02 <sup>b</sup>	1.22 ± 0.02 <sup>f</sup>	<0.001
SCFA <sup>4</sup>	8.67 ± 0.05 <sup>b</sup>	8.37 ± 0.05 <sup>c</sup>	7.33 ± 0.05 <sup>e</sup>	8.49 ± 0.03 <sup>c</sup>	8.88 ± 0.03 <sup>a</sup>	8.02 ± 0.03 <sup>d</sup>	<0.001
MCFA <sup>5</sup>	20.13 ± 0.18 <sup>bc</sup>	21.17 ± 0.18 <sup>a</sup>	19.9 ± 0.19 <sup>c</sup>	18.75 ± 0.11 <sup>d</sup>	20.49 ± 0.11 <sup>b</sup>	20.16 ± 0.11 <sup>c</sup>	<0.001
LCFA <sup>6</sup>	65.28 ± 0.26 <sup>c</sup>	65.27 ± 0.26 <sup>c</sup>	65.96 ± 0.28 <sup>b</sup>	68.0 ± 0.17 <sup>a</sup>	67.8 ± 0.17 <sup>a</sup>	66.37 ± 0.17 <sup>b</sup>	<0.001

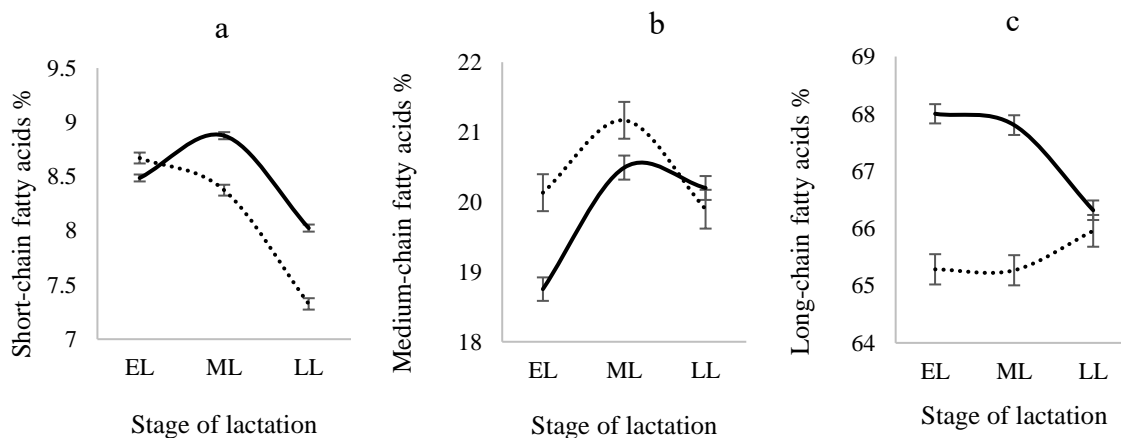
<sup>1</sup> SFA = saturated fatty acids; <sup>2</sup> UFA = unsaturated fatty acid; <sup>3</sup> PUFA = polyunsaturated fatty acid; <sup>4</sup> SCFA = short-chain fatty acids (sum of C4:0, C6:0 and C8:0); <sup>5</sup> MCFA = medium-chain fatty acids (sum of C10:0, C12:0 and C14:0); <sup>6</sup> LCFA = long-chain fatty acids (sum of C16:0, C18:0, C18:1 *cis*-9 and Omega6). <sup>7</sup> SOL = stage of lactation (early, mid and late). <sup>a, b, c, d, e, f</sup> Means with different superscripts across milking frequency and stage of lactation are significantly different (p < 0.05)

Figure 3.3 shows the proportions of major groups of fatty acids at the three stages of lactation. In both milking frequencies, the proportion of SFA was higher ( $p < 0.05$ ) in mid-lactation compared with early and late lactation. The proportion of UFA followed an opposite pattern to the proportion of SFA throughout lactation, with the proportion of PUFA being lowest ( $p < 0.05$ ) in cows milked OAD and highest in cows milked TAD in mid-lactation.



**Figure 3.3.** Proportions of (a) saturated fatty acids, (b) unsaturated fatty acids, and (c) polyunsaturated fatty in cows milked OAD (...) and TAD (—) during the 2020–2021 production season.

EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show the standard errors. The  $p$ -values for the interaction between milking frequency and stage of lactation were <0.0001 for saturated, unsaturated, and polyunsaturated fatty acids.



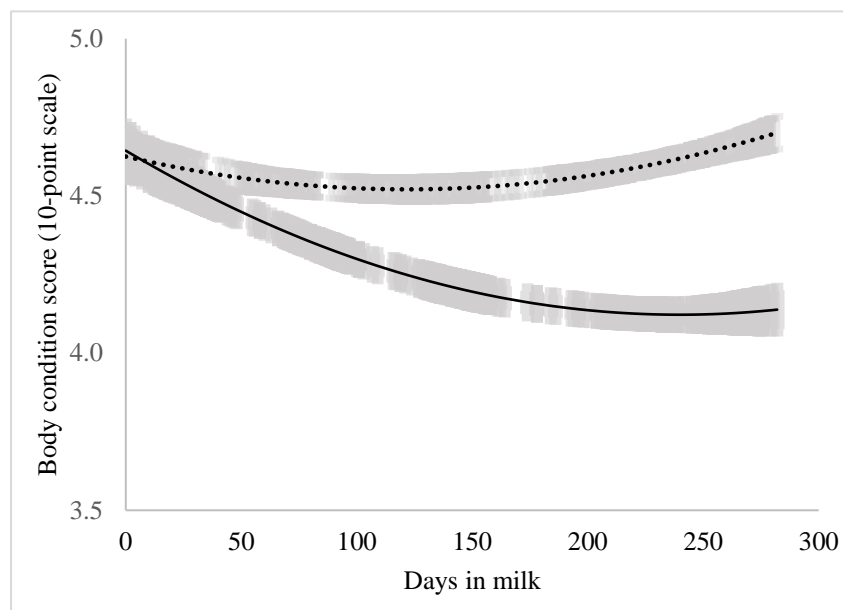
**Figure 3.4.** Proportions of (a) short-chain fatty acids, (b) medium-chain fatty acids, (c) and long-chain fatty acids in cows milked OAD (...) and TAD (—) during the 2020–2021 production season.

EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show the standard errors. The  $p$ -values for the interaction between milking frequency and stage of lactation were <0.0001 for short-chain, medium-chain, and long-chain fatty acids.

The proportions of SCFA, MCFA, and LCFA in milk fat at the three stages of lactation are shown in Figure 3.4. Stages of lactation significantly ( $p < 0.05$ ) affected the proportions of SCFA, MCFA, and LCFA.

For cows milked OAD, the proportions of SCFA were higher in early lactation and then decreased throughout lactation, while the proportion of MCFA peaked in mid-lactation. For cows milked TAD, the proportion of SCFA and MCFA increased from early lactation, peaked in mid-lactation, and decreased in late lactation. In cows milked OAD, the proportion of LCFA increased from early to late lactation and, in cows milked TAD, the proportion of LCFA decreased from early to late lactation.

Cows milked OAD had a higher BCS than cows milked TAD throughout the 2020–2021 production season (Figure 3.5). Cows milked OAD showed a slight change in BCS throughout lactation, whereas in cows milked TAD, BCS decreased as lactation progressed.



**Figure 3.5.** Body condition score of cows milked OAD (...) and TAD (—) on a 10-point scale during the 2020–2021 production season.

Grey shading indicates 95% confidence intervals.

### 3.5. Discussion

In New Zealand, TAD milking has been standard practice, but the percentage of farms milking TAD for the full season is now only 45% [25]. Once-a-day milking is practiced either in the long term or in the short term to address adverse conditions, often feed

shortages, and/or to overcome staff shortages. It is accepted that OAD milking reduces cow milk yield. In this study, cows milked OAD produced a lower daily milk yield compared with cows milked TAD. Lower milk yield led to higher percentages of fat and protein in milk from OAD milking. Once-a-day milking also affected the fatty acid composition of milk, with a lower proportion of SFA compared with cows milked TAD. Additionally, the BCS of cows milked OAD was higher than the BCS of cows milked TAD. The changes in the milk and its composition in the present study are consistent with Delamaire and Guinard-Flament [26] as well as Pulido et al. [27].

### ***3.5.1. The effect of once-a-day and twice-a-day milking on milk production and gross milk composition***

Once-a-day milking could reduce milk yield by a range of 22–50% [14]. The reduction in daily milk yield could be driven by several physiological processes. Cows milked OAD also had a higher loss of udder capacity due to an increased rate of loss in secretory cells [28]. The physiological effects were likely caused by changes in gene expression, with Grala et al. [29] showing that the expression of milk synthesis genes was lower in cows milked OAD and Littlejohn et al. [30] found differences in the expressions of genes responsible for milk production such as apoptotic signalling, mechanical stress, epithelial tight junction synthesis and milk synthesis.

Higher percentages of fat and protein in cows milked OAD compared with cows milked TAD are in agreement with Tong et al. [12], Rémond et al. [15], and Clark et al. [16]. In cows milked OAD, changes in the percentages of fat and protein may be due to the increased permeability of the tight junction between mammary epithelial cells leading to the exchange of milk and interstitial fluid [13,31]. This leakage of milk from mammary cells could create a higher percentage of milk solids [14]. Farr et al. [32] reported that in the long term, OAD milking inhibited the synthetic ability of the secretory tissues in the mammary gland and lowered the level of mammary enzymes. The lower percentage of lactose in cows milked OAD could be due to the efflux of lactose through the tight junction of the mammary gland [13]. Because lactose is an osmotically active particle that draws water, cows producing more milk will have a higher lactose percentage [13]. This is consistent with the effect observed in this study.

### 3.5.2. *The effect of once-a-day and twice-a-day milking on fatty acid composition*

The lower proportion of SFA in cows milked OAD, compared with cows milked TAD, is consistent with the findings of Dutreuil et al. [33], Ferlay et al. [34] and Pulido et al. [27]. The higher proportion of UFA in milk from cows milked OAD, compared with cows milked TAD agrees with Dutreuil et al. [33]. The lower proportion of SFA in cows milked OAD compared with cows milked TAD may be due to the lower mobilization of long-chain saturated fatty acids (C16:0 and C18:0) from adipose tissues into milk and lower synthesis of SCFA (C4:0 and C6:0). The synthesis and absorption of fatty acids are affected by the energy balance of cows [9,35], which is linked to milking frequency. Generally, in early lactation, cows have just calved and will be in a negative energy balance. In this study, it seems that OAD milking allowed the cows to recover their energy balance faster. Cows milked OAD were likely to be in an improved energy balance compared with cows milked TAD as the BCS of cows milked OAD was higher than the BCS of cows milked TAD throughout lactation. This is consistent with Rémond et al. [36], Patton et al. [37], and McNamara et al. [38]. When cows are in an improved energy balance, the utilization of LCFA from the adipose tissue could be lower and the synthesis of SCFA and MCFA could be higher. Higher absorption of LCFA and inhibition of *de novo* synthesised fatty acids are indications of negative energy balance in dairy cows [9,39]. Generally, SCFA and MCFA, from fatty acids C4:0 to C14:0 and some of C16:0, are produced by *de novo* synthesis in the mammary gland, while some of C16:0 and the rest of LCFA are absorbed from feed and body fat reserves [40].

The proportion of SCFA, mainly C4:0 and C6:0, was lower in cows milked OAD. Short-chain fatty acids are the intermediate products released by the acyl transferase enzyme, which adds two carbon units from malonyl-CoA until the chain length reaches C16 [41]. Delamaire and Guinard-Flament [26] reported the lower proportion of *de novo* synthesised fatty acids could be due to a decline in the uptake of acetate and  $\beta$ -hydroxybutyrate (precursors of *de novo* synthesis) by the mammary gland. However, in this study, the proportions of the majority of *de novo* synthesised fatty acids did not reduce, except the proportions of C4:0 and C6:0. It is possible that OAD milking could have reduced the activity of this enzyme due to the physiological changes in the mammary gland which could result in the lower proportion of SCFA. However, the consistency of MCFA suggests that acyl transferase elongation is still occurring to the same extent in

cows milked OAD. Further studies should be carried out to study enzyme activity on fatty acid synthesis in cows milked OAD.

When the cows are in negative energy balance, the absorption of LCFA is mainly from adipose tissue [39,42]. Oleic acid is a primary fatty acid in adipocytes, so it is to be expected that its proportion will be higher in milk when cows are in negative energy balance [35,43]. A lower proportion of oleic acid (C18:1 *cis*-9) in milk from cows milked OAD compared with cows milked TAD also shows the reduced use of body reserves and improved energy balance in these cows. The higher proportions of *de novo* synthesised fatty acids (from C8:0 to C14:0) in cows milked OAD also confirm the improved energy balance of these animals. In this study, the cows milked TAD were in negative energy balance compared with cows milked OAD, which led to a higher proportion of LCFA [9]. This higher uptake of LCFA may inhibit the activity of the acetyl-CoA carboxylase enzyme which converts acetyl-CoA into malonyl-CoA during the process of *de novo* synthesis [44]. This inhibitory action could be a reason for lower proportions of *de novo* synthesised fatty acids (C8:0 to C14:0) in milk from TAD milking.

In a short-term trial with Holstein cows in early lactation, Delamaire and Guinard-Flament [26] reported lower levels of *de novo* synthesised fatty acids due to a lower mammary uptake of acetate and  $\beta$ -hydroxybutyrate when milk was stored in the udder for 24 h. When cows are milked OAD for longer periods, they may adapt physiologically to the milking frequency, with the mammary gland applying different mechanisms for nutrient extraction for different milking frequencies [26]. It has been suggested that OAD milking does not alter milk fatty acid composition in cows which are in energy balance [45]. However, in the present study, energy balance associated with milking frequencies significantly changed the fatty acid composition of cows milked OAD compared with cows milked TAD. Because of these inconsistent findings, it would be useful to undertake further research to understand more about the mammary uptake of precursors and the mechanism of fatty acid synthesis in the milk of cows milked OAD.

### ***3.5.3. The effect of the stage of lactation on milk production and gross milk composition***

Daily yields of milk, fat, protein, and lactose decreased linearly as lactation progressed in both milking frequencies. Similar results were observed by Auld et al. [46] in cows milked TAD under New Zealand grazing conditions.

Cows milked OAD showed reduced daily yields of milk, fat, protein, and lactose compared with cows milked TAD throughout lactation. In cows milked OAD, the reduction in daily yields of milk, protein, and lactose was significantly greater in early lactation. The reduction in daily fat yield was significantly higher in late lactation. Similarly, Stelwagen and Knight [31] reported a greater reduction in yield in early lactation than late lactation in cows milked OAD. The reduction in daily milk yield in cows milked OAD was lower in mid-lactation than in early and late lactation. Daily milk yield reductions of 40% and 29% were reported in early-lactation in cows milked OAD by Rémond et al. [47] in a 6-week and O'Brien et al. [20] in a 10-week trial, respectively. In short-term lactation studies, greater reductions (21–22%) in milk production were observed in mid-lactation relative to late lactation [28,48,49]. In this study, daily milk yield reduction in late lactation was two-fold that of Davis et al. [50], who reported a 13% yield reduction in late lactation in a 7-day OAD milking trial. This higher reduction in OAD milking is in agreement with Stelwagen et al. [14], who stated that pasture-based seasonal farming with limited supplementary feeding could have a greater reduction in milk yield in late lactation due to lower dry matter intake. These findings suggest that the reduction in milk production may depend on the stage of lactation.

The fat and protein percentages significantly increased, and the lactose percentage decreased with advancing lactation in both OAD and TAD milking. Similar results were observed by Auldist et al. [46] in cows milked TAD. The increasing concentration of milk solids as lactation progressed may be due to a concentrating effect created by decreasing milk volume [46]. In early lactation, the percentages of fat and protein were 13% and 7% higher, respectively, and lactose percentage was 4% lower in cows milked OAD than in cows milked TAD. During mid-lactation, fat and protein percentages were 22% and 11% greater, respectively, and lactose percentage was 6% lower in cows milked OAD than in cows milked TAD. Similarly, Pomies et al. [19] reported higher fat and protein percentages in mid-lactation in cows milked OAD, but the values were lower (7% and 5%, respectively) than in the current study. Similar trends were observed in late lactation, which were in agreement with Lacy-Hulbert et al. [50], O'Brien et al. [20], and Ferris et al. [51]. However, the percentage of fat increased by 26% in late lactation, which was the highest rise in cows milked OAD compared with cows milked TAD in the production season and the greatest of the above studies. Modifications in milk composition in OAD milking compared with TAD have been suggested to occur due to the increased exchange

of milk and extra-cellular fluid through the increasingly permeable mammary gland membrane [13].

#### **3.5.4. The effect of stage of lactation on fatty acid composition**

In the present study, results obtained on the effect of stages of lactation on fatty acid composition are in agreement with Back and Thomson [8], Kgwatalala et al. [52], and Stoop et al. [9] for cows milked TAD. Trends followed by proportions of SFA and UFA in cows milked OAD were similar to cows milked TAD and in agreement with the above studies. The proportion of SFA was significantly higher in mid-lactation than in early-lactation and late-lactation in both milking frequencies. The proportion of UFA followed the opposite trend to that of the proportion of SFA. In early lactation, the lower proportion of SFA suggests that the cow might be in a negative energy balance [42] due to high milk production and inadequate dry matter intake. In mid-lactation, the cows tend to increase their energy balance, which could lead to an increase in the proportion of SFA. In early lactation, due to negative energy balance, higher mobilisation of oleic acid could take place, which could have led to a higher proportion of oleic acid [52] and UFA.

The proportion of SCFA decreased and the proportion of LCFA increased as lactation progressed in cows milked OAD. The reason behind the lower proportion of SCFA in mid-lactation in cows milked OAD is not clear although the energy balance (linked to BCS) of the cows milked OAD was better in mid-lactation than early lactation. Proportions of SCFA and MCFA were higher in mid-lactation than early and late lactation in cows milked TAD, which is in agreement with Stoop et al. [9], Gross et al. [35], and Schwendel et al. [53]. In early lactation, cows milked OAD may be in lower negative energy balance compared with cows milked TAD and they can continue the *de novo* synthesis in the mammary gland without inhibition by the higher absorption of LCFA (the proportion of LCFA was lower in EL). In cows milked TAD, due to negative energy balance in early lactation, the *de novo* synthesis of SCFA and MCFA may be reduced. The higher proportion of SCFA, mainly C4:0 and C6:0, in mid- and late lactation in cows milked TAD compared with cows milked OAD could be explained by the variation in their synthesis pathway [44] and these pathways are less affected by the inhibitory effect of LCFA. C4:0 is produced by two pathways: directly from preform 4 carbon  $\beta$ -hydroxybutyrate and by the malonyl-CoA-independent pathway ( $\beta$ -reduction pathway) by condensation of acetyl units [44]. The synthesis of C6:0 is also less affected by the

inhibition effect due to the addition of only one acetyl unit by malonyl-CoA (longer acyl chains are more affected) [44]. The results of this study reveal that the effect of milking frequencies and stages of lactation on fatty acid composition could be connected to the energy balance of cows [52].

The proportions of individual fatty acids in cow milk affect butter hardness and cheese coagulation properties. Cows milked OAD tend to produce harder butter (lower spreadability) compared with cows milked TAD due to higher proportions of fatty acids from C8:0 to C14:0 and lower proportion of C18:1. MacGibbon [54] reported that these proportions of fatty acids would increase the solid fat content of milk fat at a temperature of 10°C (SCF<sub>10</sub>), which increases the hardness of the butter. When the SCF<sub>10</sub> is lower, the butter will be soft; and when it is higher, the butter will be hard [54]. However, a lower proportion of C16:0 in cows milked OAD tends to decrease the hardness of the butter [55]. Overall, there is probably little change in the hardness of the fat and subsequently little change in the spreadability of butter. The higher proportions of C8:0, C10:0 and C12:0 and lower proportion of C18:1 *cis*-9 in cows milked OAD may affect rennet coagulation properties as measured using a Formagraph. In particular, it may decrease the curd firming time, i.e., a shorter K<sub>20</sub> time, and increase curd firmness after 60 min (A<sub>60</sub> parameter) [2]. While the fatty acid composition due to OAD milking may affect the cheese yield compared with TAD milking, Auld et al. [2] reported that the relationships between fatty acid composition and cheese coagulation parameters were too weak to make consistent predictions. Of more commercial interest, the higher percentages of fat and protein in milk from cows milked OAD are likely to increase the weight of curd produced from a given volume of milk in the cheese-making process [3]. This would result in an increase in the capacity of an existing cheese plant, but this must be traded off against the reduced fat and protein yields per cow.

In New Zealand, the seasonal calving is synchronised with the season of the year, pasture growth, and stages of lactation. It is difficult to distinguish to what extent the season, stage of lactation, or the interaction of both affect the proportions of fatty acids in milk [51]. Auld et al. [46] found a significant interaction between the season of the year and the stage of lactation for milk fatty acid composition. However, in the present study, only the effect of stages of lactation on fatty acid composition was studied and the season of the year was not investigated. In addition, this study discusses the *de novo* synthesised fatty acids more as they are less affected by the feed, and mainly a precursor- and enzyme-

dependent pathway [40] in the mammary gland. One of the factors which affect the proportions of these fatty acids is the energy balance of cows, and the energy balance is affected by milking frequency and the level of feed intake.

### 3.6. Conclusions

Cows milked OAD had a significantly lower daily yield of milk and higher percentages of fat and protein compared with cows milked TAD. Cows milked OAD produced milk with significantly higher proportions of *de novo* synthesised fatty acids and a lower proportion of LCFA compared with cows milked TAD. These findings suggest that the fatty acid composition of milk is significantly affected by milking frequency in relation to the energy balance of cows. However, the confounding effect of feed was the limitation of this study. The findings of this study would be useful in selecting OAD milk for processing into dairy products. Further research would be useful to explore the effect of OAD milking on the mechanism of fatty acid synthesis in cows milked OAD.

### References

1. Dalgleish, D.G. Bovine milk protein properties and the manufacturing quality of milk. *Livest Prod Sci.* **1993**, *35*, 75–93, [https://doi.org/10.1016/0301-6226\(93\)90183-I](https://doi.org/10.1016/0301-6226(93)90183-I).
2. Auldist, M.J.; Johnston, K.A.; White, N.J.; Fitzsimons, W.P.; Boland, M.J. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *J. Dairy Sci.* **2004**, *71*, 51–57, <https://doi.org/10.1017/s0022029903006575>.
3. Amenu, B.; Deeth, H.C. The impact of milk composition on cheddar cheese manufacture. *Aust. J. Dairy Technol.* **2007**, *62*, 171–184.
4. MacGibbon, A.K.H.; Taylor, M.M. Composition and structure of bovine milk lipids. In *Advanced Dairy Chemistry*, 3rd ed.; Fox, P.F., McSweeney, P.L.H., Eds.; Springer: New York, NY, USA, 2006; Volume 2, pp. 1–42.
5. Grummer, R.R. Effect of feed on the composition of milk fat. *J. Dairy Sci.* **1991**, *74*, 3244–3257, [https://doi.org/10.3168/jds.S0022-0302\(91\)78510-X](https://doi.org/10.3168/jds.S0022-0302(91)78510-X).
6. Bauman, D.E.; Griinari, J.M. Nutritional regulation of milk fat synthesis. *Annu. Rev. Nutr.* **2003**, *23*, 203–227, <https://doi.org/10.1146/annurev.nutr.23.011702.073408>.
7. Palladino, R.A.; Buckley, F.; Prendiville, R.; Murphy, J.J.; Callan, J.; Kenny, D.A. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid

- on milk fatty acid composition under grazing conditions. *J. Dairy Sci.* **2010**, *93*, 2176–2184, <https://doi.org/10.3168/jds.2009-2453>.
8. Back, P.J.; Thomson, N.A. Exploiting cow genotype to increase milk value through production of minor milk components. *Proc. N. Z. Soc. Anim. Prod.* **2005**, *65*, 53–58.
  9. Stoop, W.M.; Bovenhuis, H.; Heck, J.M.L.; van Arendonk, J.A.M. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *J. Dairy Sci.* **2009**, *92*, 1469–1478, <https://doi.org/10.3168/jds.2008-1468>.
  10. Arnould, V.M.R.; Hammami, H.; Soyeurt, H.; Gengler, N. Short communication: Genetic variation of saturated fatty acids in Holsteins in the Walloon region of Belgium. *J. Dairy Sci.* **2010**, *93*, 4391–4397, <https://doi.org/10.3168/jds.2010-3049>.
  11. Lucey, J. Cheesemaking from grass based seasonal milk and problems associated with late-lactation milk. *Int. J. Dairy Technol.* **1996**, *49*, 59–64, <https://doi.org/10.1111/j.1471-0307.1996.tb02491.x>.
  12. Tong, M.J.; Clark, D.A.; Cooper, C.V. Once-a-day milking: Possible and profitable? *Proc. N. Z. Grassl. Assoc.* **2002**, *64*, 33–37, <https://doi.org/10.33584/jnzg.2002.64.2470>.
  13. Davis, S.R.; Farr, V.C.; Stelwagen, K. Regulation of yield loss and milk composition during once-daily milking: A review. *Livest. Prod. Sci.* **1999**, *59*, 77–94, [https://doi.org/10.1016/S0301-6226\(98\)00204-8](https://doi.org/10.1016/S0301-6226(98)00204-8).
  14. Stelwagen, K.; Phyn, C.V.V.; Davis, S.R.; Guinard-Flament, J.; Pomiès, D.; Roche, J.R.; Kay, J.K. Invited review: Reduced milking frequency. Milk production and management implications. *J. Dairy Sci.* **2013**, *96*, 1–13, <https://doi.org/10.3168/jds.2012-6074>.
  15. Rémond, B.; Pomiès, D.; Dupont, D.; Chilliard, Y. Once-a-day milking of multiparous Holstein cows throughout the entire lactation: Milk yield and composition, and nutritional status. *Anim. Res.* **2004**, *53*, 201–212, <https://doi.org/10.1051/animres:2004014>.
  16. Clark, D.A.; Phyn, C.V.C.; Tong, M.J.; Collis, S.J.; Dalley, D.E. A systems comparison of once-versus twice daily milking of pastured dairy cows. *J. Dairy Sci.* **2006**, *89*, 1854–1862, [https://doi.org/10.3168/jds.S0022-0302\(06\)72254-8](https://doi.org/10.3168/jds.S0022-0302(06)72254-8).
  17. Davis, S.R.; Turner, S.A.; Obolonkin, V.; Tiplady, K.; Spelman, R.J.; Phyn, C.V. Lactation traits associated with short-and long-term once-daily milking performance

- in New Zealand crossbred dairy cattle. *J. Dairy Sci.* **2015**, *98*, 6094–6107, <https://doi.org/10.3168/jds.2014-8948>.
18. Martin, B.; Pomies, D.; Pradel, P.; Verdier-Metz, I.; Rémond, B. Yield and sensory properties of cheese made with milk from Holstein or Montbéliarde cows milked twice or once daily. *J. Dairy Sci.* **2009**, *92*, 4730–4737.
  19. Pomies, D.; Martin, B.; Chilliard, Y.; Pradel, P.; Rémond, B. Once-a-day milking of Holstein and Montbéliarde cows for 7 weeks in mid-lactation. *Animal* **2007**, *1*, 1497–1505, <https://doi.org/10.1017/S1751731107000778>.
  20. O'Brien, B.; Ryan, G.; Meaney, W.J.; McDonagh, D.; Kelly, A. Effect of frequency of milking on yield, composition, and processing quality of milk. *J. Dairy Res.* **2002**, *69*, 367–374, <https://doi.org/10.1017/S0022029902005605>.
  21. Correa-Luna, M.; Donaghy, D.; Kemp, P.; Schutz, M.; López-Villalobos, N. Efficiency of crude protein utilisation in grazing dairy cows: A case study comparing two production systems differing in intensification level in New Zealand. *Animals* **2020**, *10*, 1036. <https://doi.org/10.3390/ani10061036>.
  22. Corson, D.C.; Waghorn, G.C.; Ulyatt, M.J.; Lee, J. NIRS: Forage analysis and livestock feeding. *Proc. N. Z. Grassl. Assoc.* **1999**, *61*, 127–132.
  23. Sukhija, P.S.; Palmquist, D.L. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J. Agric. Food Chem.* **1988**, *36*, 1202–1206, <https://doi.org/10.1021/jf00084a019>.
  24. Dairy NZ. Body Condition Scoring Made Easy, 2004. Available online: <https://www.dairynz.co.nz/media/5790783/body-condition-scoring-made-easy-booklet.pdf> (accessed on 16 September 2021).
  25. Dairy NZ. Milking is the Largest Labour Requirement on a Dairy Farm and the Timing of Milking Defines the Whole Structure of the Day, 2021. Available online: [https://www.dairynz.co.nz/milking/milking-intervals/#:~:text=The%20three%20main%20examples%20of,\(1%20milking%20per%20day\)](https://www.dairynz.co.nz/milking/milking-intervals/#:~:text=The%20three%20main%20examples%20of,(1%20milking%20per%20day)) (accessed on 7 October 2021).
  26. Delamaire, E.; Guinard-Flament, J. Increasing milking intervals decreases the mammary blood flow and mammary uptake of nutrients in dairy cows. *J. Dairy Sci.* **2006**, *89*, 3439–3446, [https://doi.org/10.3168/jds.S0022-0302\(06\)72381-5](https://doi.org/10.3168/jds.S0022-0302(06)72381-5).
  27. Pulido, E.; Fernández, M.; Prieto, N.; Baldwin, R.L.; Andrés, S.; López, S.; Giráldez, F.J. Effect of milking frequency and  $\alpha$ -tocopherol plus selenium supplementation on

- sheep milk lipid composition and oxidative stability. *J. Dairy Sci.* **2019**, *102*, 3097–3109, <https://doi.org/10.3168/jds.2018-15456>.
28. Carruthers, V.R.; Davis, S.R.; Bryant, A.M.; Henderson, H.V.; Morris, C.A.; Copeman, P.J. Response of Jersey and Friesian cows to once a day milking and prediction of response based on udder characteristics and milk composition. *J. Dairy Sci.* **1993**, *60*, 1–11, <https://doi.org/10.1017/S0022029900027291>.
  29. Grala, T.M.; Phyn, C.V.; Kay, J.K.; Rius, A.G.; Littlejohn, M.D.; Snell, R.G.; Roche, J.R. Temporary alterations to milking frequency, immediately post-calving, modified the expression of genes regulating milk synthesis and apoptosis in the bovine mammary gland. *Proc. N. Z. Soc. Anim. Prod.* **2011**, *71*, 3–8.
  30. Littlejohn, M.D.; Walker, C.G.; Ward, H.E.; Lehnert, K.B.; Snell, R.G.; Verkerk, G.A.; Spelman, R.J.; Clark, D.A.; Davis, S.R. Effects of reduced frequency of milk removal on gene expression in the bovine mammary gland. *Physiol. Genomics* **2010**, *41*, 21–32, <https://doi.org/10.1152/physiolgenomics.00108.2009>.
  31. Stelwagen, K.; Knight, C.H. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. *J. Dairy Sci.* **1997**, *64*, 487–494, <https://doi.org/10.1017/s0022029997002458>.
  32. Farr, V.C.; Stelwagen, K.; Kerr, M.A.; Davis, S.R. Effect of once daily milking (ODM) on enzyme activities in the bovine mammary gland. *Proc. N. Z. Soc. Anim. Prod.* **1995**, *55*, 12–13.
  33. Dutreuil, M.; Guinard-Flament, J.; Boutinaud, M.; Hurtaud, C. Effect of duration of milk accumulation in the udder on milk composition, especially on milk fat globule. *J. Dairy Sci.* **2016**, *99*, 3934–3944, <https://doi.org/10.3168/jds.2015-10002>.
  34. Ferlay, A.; Martin, B.; Lerch, S.; Gobert, M.; Pradel, P.; Chilliard, Y. Effects of supplementation of maize silage diets with extruded linseed, vitamin E and plant extracts rich in polyphenols, and morning vs evening milking on milk fatty acid profiles in Holstein and Montbéliarde cows. *Animal* **2010**, *4*, 627–640, <https://doi.org/10.1017/S1751731109991224>.
  35. Gross, J.; van Dorland, H.A.; Bruckmaier, R.M.; Schwarz, F.J. Milk fatty acid profile related to energy balance in dairy cows. *J. Dairy Sci.* **2011**, *78*, 479–488, <https://doi.org/10.1017/S0022029911000550>.
  36. Rémond, B.; Pomiès, D. Once-daily milking of dairy cows: A review of recent French experiments. *Anim. Res.* **2005**, *54*, 427–442, <https://doi.org/10.1051/animres:2005040>.

37. Patton, J.; Kenny, D.A.; Mee, J.F.; O'mara, F.P.; Wathes, D.C.; Cook, M.; Murphy, J.J. Effect of milking frequency and diet on milk production, energy balance, and reproduction in dairy cows. *J. Dairy Sci.* **2006**, *89*, 1478–1487, [https://doi.org/10.3168/jds.S0022-0302\(06\)72215-9](https://doi.org/10.3168/jds.S0022-0302(06)72215-9).
38. McNamara, S.; Murphy, J.J.; O'mara, F.P.; Rath, M.; Mee, J.F. Effect of milking frequency in early lactation on energy metabolism, milk production and reproductive performance of dairy cows. *Livest. Sci.* **2008**, *117*: 70–78, [https://doi.org/10.3168/jds.S0022-0302\(06\)72215-9](https://doi.org/10.3168/jds.S0022-0302(06)72215-9).
39. Bastin, C.; Gengler, N.; Soyeurt, H. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. *J. Dairy Sci.* **2011**, *94*, 4152–4163, <https://doi.org/https://doi.org/10.3168/jds.2010-4108>.
40. MacGibbon, A.K.H. Composition and Structure of Bovine Milk Lipids. In *Advanced Dairy Chemistry*, 4th ed.; McSweeney, P.L.H., Fox, P.F., O'Mahony, J.A., Eds.; Springer: New York, NY, USA, 2020; Volume 2, pp. 1–32.
41. Palmquist, D.L.; Harvatine, K.J. Origin of fatty acids and influence of nutritional factors on milk fat. In *Advanced Dairy Chemistry*, 4th ed.; McSweeney, P.L.H., Fox, P.F., O'Mahony, J.A., Eds.; Springer: New York, NY, USA, 2020; Volume 2, pp. 33–66.
42. Vranković, L.; Aladrović, J.; Octenjask, D.; Bijelić, D.; Cvetnić, L.; Stojević, Z. Milk fatty acid composition as an indicator of energy status in Holstein dairy cows. *Arch. Anim. Breed.* **2017**, *60*, 205–212, <https://doi.org/10.5194/aab-60-205-2017>.
43. Rukkamsuk, T.; Geelen, M.J.H.; Kruip, T.A.M.; Wensing, T. Interrelation of fatty acid composition in adipose tissue, serum, and liver of dairy cows during the development of fatty liver postpartum. *J. Dairy Sci.* **2000**, *83*, 52–59, [https://doi.org/10.3168/jds.S0022-0302\(00\)74854-5](https://doi.org/10.3168/jds.S0022-0302(00)74854-5).
44. Palmquist, D.L.; Beaulieu, A.D.; Barbano, D.M. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* **1993**, *76*, 1753–1771, [https://doi.org/10.3168/jds.S0022-0302\(93\)77508-6](https://doi.org/10.3168/jds.S0022-0302(93)77508-6).
45. Chilliard, Y.; Pomies, D.; Pradel, P.; Rémond, B. Once daily milking does not change milk fatty acid profile in cows in equilibrated energy balance. In *Proceedings of the 57th Annual Meeting of the European Association for Animal Production (EAAP), Antalya, Turkey, 17–20 September 2006*; Wageningen Academic Publishers: 2006,

46. Auldism, M.J.; Walsh, B.J.; Thomson, N.A. Seasonal and lactational influences on bovine milk composition in New Zealand. *J. Dairy Res.* **1998**, *65*, 401–411, <https://doi.org/10.1017/s0022029998002970>.
47. Rémond, B.; Coulon, J.B.; Nicloux, M.; Levieux, D. Effect of temporary once-daily milking in early lactation on milk production and nutritional status of dairy cows. *Ann. Zootech.* **1999**, *48*, 341–352, <https://hal.archives-ouvertes.fr/hal-00889807>.
48. Davis, S.R.; Farr, V.C.; Stelwagen, K. Once daily milking of dairy cows: An appraisal. *Proc. N. Z. Soc. Anim. Prod.* **1998**, *58*, 36–40.
49. Davis, S.; McNaughton, L.; Bracefield, G.; Sanders, K.; Spelman, R. Variation in milk yield response to once-daily milking in Friesian-Jersey crossbred cattle. *J. Dairy Sci.* **2006**, *89*, 146–147.
50. Lacy-Hulbert, S.J.; Woolford, M.W.; Nicholas, G.D.; Prosser, C.G.; Stelwagen, K. Effect of milking frequency and pasture intake on milk yield and composition of late lactation cows. *J. Dairy Sci.* **1999**, *82*, 1232–1239, [https://doi.org/10.3168/jds.S0022-0302\(99\)75346-4](https://doi.org/10.3168/jds.S0022-0302(99)75346-4).
51. Ferris, C.P.; Frost, J.P.; Mayne, C.S.; McCoy, M.A.; Kilpatrick, D.J. A comparison of the direct and residual response of dairy cows to once or twice-daily milking, in late lactation. *Livest. Sci.* **2008**, *114*, 305–314, <https://doi.org/10.1016/j.livsci.2007.05.015>.
52. Kgwatalala, P.M.; Ibeagha-Awemu, E.M.; Mustafa, A.F.; Zhao, X. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. *Anim. Genet.* **2009**, *40*, 609–615, <https://doi.org/10.1111/j.1365-2052.2009.01887.x>.
53. Schwendel, B.H.; Morel, P.C.; Wester, T.J.; Tavendale, M.H.; Deadman, C.; Fong, B.; Shadbolt, N.M.; Thatcher, A.; Otter, D.E. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. *J. Dairy Sci.* **2015**, *98*, 1411–1425, <https://doi.org/10.3168/jds.2014-8322>.
54. MacGibbon, A.K. Herd-to-herd variations in the properties of milkfat. *Proc. N. Z. Soc. Anim. Prod.* **1996**, *56*, 224–227.
55. Mackle, T.R.; Petch, S.F.; Bryant, A.M.; Auldism, M.J.; Henderson, H.V.; MacGibbon, A.K.H. Variation in the characteristics of milkfat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *N. Z. J. Agric. Res.* **1997**, *40*, 349–359, <https://doi.org/10.1080/00288233.1997.9513254>.



### Appendix 1 (Supplementary information for Chapter 3)

**Appendix 1 Table 1.** Chemical composition of individual feed offered at No. 1 Dairy (once-a-day milking) during the sampling season in early, mid, and late lactations in the 2020-2021 production season.

Lactation stage	Early					Mid			Late			
Item	Pasture	Herb-mix crop <sup>1</sup>	Maize silage	DDG <sup>2</sup>	Tapioca	Pasture	Herb-mix crop	Pasture	Herb-mix crop	Tapioca	Corn gluten feed pallets	Lucerne baleage
CP <sup>3</sup> % of DM	23.1	18.0	9.0	31.9	5.2	20.4	16.9	23.0	20.4	4.6	17.2	30.7
NDF <sup>4</sup> % of DM	40.3	33.2	47.2	43.2	29.1	41.2	30.9	50.3	36.0	27.0	31.4	40.1
ADF <sup>5</sup> % of DM	21.2	21.2	33.6	9.6	21.6	20.9	18.8	27.2	21.2	23.3	19.8	32.3
Lipid % of DM	4.4	3.7	3.3	8.31	0.1	4.6	4.9	4.9	4.5	-	6.2	5.0
SSS <sup>6</sup> % of DM	13.4	13.1	28.3	12.8	40.7	15.1	11.1	9.8	5.6	35.4	7.0	1.0
ME <sup>7</sup> MJ ME/kg DM	13.0	11.9	9.8	7.9	11.3	11.8	13.0	9.3	10.8	10.8	7.8	10.9

<sup>1</sup> Herb-mix comprises chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and red clover (*Trifolium pratense*); <sup>2</sup> Dried distillers grains; <sup>3</sup> CP= crude protein; <sup>4</sup> NDF= neutral detergent fibre; <sup>5</sup> ADF= acid detergent fibre; <sup>6</sup> SSS= soluble sugars and starch; <sup>7</sup> ME= metabolisable energy; Baleage is dried forage, developed from the process of bailing the forage.

**Appendix 1 Table 2.** Chemical composition individual feed offered at No. 4 Dairy (twice-a-day milking) during the sampling season in early, mid, and late lactations in the 2020-2021 production season.

Lactation stage	Early				Mid				Late				
	Pasture	Hay	Maize silage	Soy meal	Pasture	Grass baleage	Maize	DDG <sup>1</sup>	Pasture	Straw	Grass baleage	Corn gluten feed pallet	Maize silage
CP <sup>2</sup> % of DM	22.4	13.0	6.7	35.2	23.1	9.7	7.7	17.5	30.4	2.7	15.9	17.1	6.7
NDF <sup>3</sup> % of DM	43.0	66.2	51.8	21.0	44.7	62.7	38.3	30.1	44.9	86.4	46.3	35.9	47.4
ADF <sup>4</sup> % of DM	21.0	39.1	20.6	13.0	21.3	37.0	29.7	18.6	20.1	54.8	29.6	21.1	29.8
Lipid % of DM	5.0	0.8	3.3	1.4	4.1	2.3	3.9	6.2	5.9	1.0	4.0	5.7	3.2
SSS <sup>5</sup> % of DM	14.1	2.2	40.5	-	12.4	13.6	38.2	4.7	8.4	3.5	4.7	2.0	32.9
ME <sup>6</sup> MJ ME/kg DM	11.4	7.3	11.3	12.8	11.0	8.0	10.2	7.6	10.9	3.5	12.4	7.2	10.2

<sup>1</sup> Dried distillers grains; <sup>2</sup> CP= crude protein; <sup>3</sup> NDF= neutral detergent fibre; <sup>4</sup> ADF= acid detergent fibre; <sup>5</sup> SSS= soluble sugars and starch; <sup>6</sup> ME= metabolisable energy; Baleage is dried forage, developed from the process of bailing the forage

## **Foreword to Chapter 4**

This chapter explored the effect of breed on fatty acid composition. First and second parity Holstein-Friesian, Holstein-Friesian  $\times$  Jersey, and Jersey cows from the No. 1 Dairy farm (once-a-day milking) and first and second parity Holstein-Friesian and Holstein-Friesian  $\times$  Jersey cows from the young mob on the No. 4 Dairy farm (twice-a-day milking) were selected for this chapter. There were no Jersey cows in the No. 4 Dairy farm, and therefore, the breed effect was evaluated with a linear mixed model that considered breed nested within the milking frequency.



## Chapter 4

### Effect of breed on the fatty acid composition of milk from dairy cows milked once and twice a day in different stages of lactation

Published as:

Sanjayanj, I.; Lopez-Villalobos, N.; Blair, H.T.; Janssen, P.W.M.; Holroyd, S.E.; MacGibbon, A.K.H. Effect of breed on the fatty acid composition of milk from dairy cows milked once and twice a day in different stages of lactation. *Dairy*. **2022**, *3*, 608–621. <https://doi.org/10.3390/dairy3030043>



#### 4.1. Abstract

The objective of this study was to evaluate the effect of breed on the overall composition and fatty acid composition of milk from cows milked once a day (OAD) and twice a day (TAD) in different stages of lactation. Milk samples were taken from 39 Holstein-Friesian (F), 27 Jersey (J), and 34 Holstein-Friesian  $\times$  Jersey (F $\times$ J) cows from a OAD milking herd and 104 F and 83 F $\times$ J cows from a TAD milking herd in early ( $49 \pm 15$  days in milk), mid- ( $129 \pm 12$  days in milk), and late ( $229 \pm 13$  days in milk) lactation. Calibration equations to predict the proportions of individual fatty acids were developed using mid-infrared spectroscopy. There was a significant interaction between breeds within the milking frequency and stage of lactation for the production traits and composition traits. Holstein-Friesian cows milked OAD produced milk with lower proportion of C18:0 in early and mid-lactation compared with F $\times$ J and J cows. Holstein-Friesian cows milked TAD produced lower proportion of C18:0 in early lactation and lower proportions of C16:0 and C18:0 in late lactation compared with F $\times$ J. Lower proportions of these fatty acids would reduce the hardness of the butter when the milk is processed. In the OAD milking herd, F cows were superior for daily milk yield compared with J cows, but Jersey cows produced significantly ( $p < 0.05$ ) higher percentage of fat and a higher proportion of C18:0. The relative proportions of C18:0 and C18:1 *cis*-9 in F and J cows milked OAD imply there is no breed effect on the activity of delta-9-desaturase, whereas stages of lactation likely have an effect. These results can be used to assist with selecting breeds and cows that are suitable for either OAD or TAD milking, allowing closer alignment with milk processing needs.

Keywords: breed; dairy cattle; fatty acid; milking frequency; New Zealand; stage of lactation

#### 4.2. Introduction

Dairy farming in New Zealand is pasture-based and spring-calving with a traditional practice of twice-a-day (TAD) milking. In recent years, some farmers have adopted once-a-day (OAD) milking due to farm management and animal welfare benefits [1,2]. Before 1960, the New Zealand dairy herd was approximately 70% Jersey (J) cows, however; post-1960, Holstein-Friesian (F) cows became very popular [3] due to their greater production of milk solids [4]. Crossbreeding between F and J breeds has been practiced since the 1960s [5]. Cross-bred cows usually have similar productive but better

reproductive performance and survival rates compared with the parent breeds due to heterosis [6–8]. The New Zealand dairy herd currently consists of 49.1% Holstein-Friesian × Jersey (F×J), 32.7% F, 8.4% J, and 9.3% other breeds and crosses [4].

Milk composition affects the processability of milk into products such as cheese and butter [9,10]. Milk solid fat content at 10°C (SFC<sub>10</sub>), which is influenced by milk fat and fatty acid composition, determines the hardness (or the spreadability) of butter. The higher the SFC<sub>10</sub>, the harder the butter. [11]. Softer butter is preferred mostly due to its ability to be easily spreadable at low temperatures.

Milk fatty acid composition is affected by genetic variation [11,12], breed [9,12,13], stage of lactation [14,15,16], diet [17,18], and season [19,20]. Metabolic disorders such as ketosis [21,22] and sub-acute ruminal acidosis [23,24] also affect the fat and fatty acid composition of milk. Among these factors, the breed has the greatest effect on fatty acid composition [25]. Milk from F cows has greater proportions of unsaturated fatty acid (UFA) and lower proportions of saturated fatty acid (SFA) relative to J cows. Milk from J cows has higher proportions of short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) compared with F cows [26].

Several studies have investigated the effects of breed on milk production [3,5,27] and fatty acid composition [4,12,25], and also the effect of stage of lactation on milk fatty acid composition [13,28,29] in cows milked TAD. Few studies have investigated the effect of breed on milk production traits for OAD and TAD milking [5,27]. However, the effect of breed on the fatty acid composition of cows milked OAD in different stages of lactation has not been reported. The objective of this study was to examine the milk composition and fatty acid composition of different breeds of cows milked OAD and TAD in different stages of lactation without control of the feed.

### **4.3. Materials and Methods**

#### ***4.3.1. Farms and cows***

The experiment took place on No.1 Dairy farm and No.4 Dairy farm (latitude: 40°22'35.1", longitude: 175°36'51.1"), Massey University, New Zealand. The soil type and climatic conditions of both farms are similar. Fresh ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture are used as a predominant feed on both farms. The No.1 Dairy farm is managed in a OAD milking system with lower supplementation

and a lower stocking rate of 2.4 cows/ha. In contrast, the No.4 Dairy farm is managed in a TAD milking system with higher supplements and a higher stocking rate of 2.6 cows/ha.

Cows on No. 1 dairy were fed approximately 8, 12, and 4.5 kg DM/cow/day pasture and 4, 4, and 3 kg DM/cow/day herb mix crop containing chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and red clover (*Trifolium pratense*) in early, mid- and late lactation respectively. The cows were fed maize silage, tapioca, and dried distillers grains in early lactation and tapioca pellets, concentrate (corn gluten feed pellets), and lucerne baleage in late lactation.

Cows on the No. 4 dairy were fed approximately 17, 17 and 10 kg DM/cow/day pasture in early, mid- and late lactation respectively. Maize silage was fed throughout the lactation. In addition, concentrate (soy meal), and dry roughage in early lactation, dried distillers grains and grass baleage in mid-lactation and concentrate (corn gluten feed pellets), straw and grass baleage in late lactation were fed as supplements. Details of feed and chemical composition of feed of the farms where this study was carried out are provided in Table 3.1 in Chapter 3. The confounding effect of feed and milking frequency was the limitation of this study as the feed provided to cows milked OAD and TAD were slightly different.

One hundred spring calving cows (39 F, 27 J, and 34 F×J) from the OAD milking herd and 187 spring calving cows (104 F and 83 F×J) from the TAD milking herd were used in this study. Cows were of first (54 cows from No.1 Dairy and 123 cows from No.4 Dairy) and second (46 cows from No.1 Dairy and 64 cows from No.4 Dairy) parity, with no clinical mastitis or metabolic diseases during the sampling period. There is a genetic connection between the two farms as there are common sires on the two farms.

#### **4.3.2. Sampling of cows**

Selected No.1 Dairy cows were milked at 6.30 am and the selected No.4 Dairy cows were milked at 5.30 am and 2.30 pm during early ( $49 \pm 15$  days in milk), mid- ( $129 \pm 12$  days in milk), and late ( $229 \pm 13$  days in milk) lactation in the 2020–2021 production season (cows were milked once in each stage of lactation). The Waikato milk meters were used to collect composite milk samples. The samples were stored in a refrigerator at 0–4°C immediately after collecting until analysis. Preservatives were not added, and all the analyses were carried out within two days of collection.

### 4.3.3. Analysis of milk samples

The percentages of fat, protein, and lactose in individual samples were determined by a Milkoscan FT1 (Foss, Hillerød, Denmark). The proportions of individual fatty acids were predicted using the calibration equations developed in FTIR calibrator software (Foss Analytical, Hillerød, Denmark) using a data set with mid-infrared spectral data and gas chromatography reference values. Milk composition was analysed by following the ISO standard of ISO 9622:2013 [IDF 141:2013].

### 4.3.4. Measurement of body condition scores of the cows

The monthly body condition score (BCS) of each cow was measured from calving to dry-off date during the 2020–2021 production season, by a single research technician using a 10-point scale [30].

### 4.3.5. Statistical analysis

Data were analysed using a MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) statistical software. The following mixed linear model was used to obtain the least-square means and the standard errors:

$$Y_{ijklmn} = \mu + M_i + B_j(M_i) + L_k + S_l + S_l B_j(M_i) + \beta_1 d_m + C_m + e_{ijklmn}$$

where

$Y_{ijklmn}$  is the observation  $n$  for any of the production traits and composition traits in milking frequency  $i$ , breed  $j$ , lactation number  $k$ , stage of lactation  $l$ , and cow  $m$ .

$\mu$  is the population mean.

$M_i$  is the fixed effect of milking frequency ( $i = \text{OAD}$  and  $\text{TAD}$ ).

$B_j(M_i)$  is the fixed effect of breed  $j$  nested in milking frequency  $i$  ( $j = \text{F}$ ,  $\text{F} \times \text{J}$ , and  $\text{J}$ ).

$L_k$  is the fixed effect of lactation number ( $k = 1^{\text{st}}$  and  $2^{\text{nd}}$  lactation).

$S_l$  is the fixed effect of the stage of lactation ( $l = \text{early}$ ,  $\text{mid}$ , and  $\text{late}$ ).

$S_l B_j(M_i)$  is the fixed effect of interaction between stages of lactation  $l$  and breed  $j$  nested in milking frequency  $i$ .

$\beta_1$  is the regression coefficient of the linear effect of deviation (days) from herd median calving date on trait  $Y$  of cow  $m$ .

$C_m$  is the random effect of cow ( $m = 1, 2 \dots 287$ ) assumed with mean zero and variance  $\sigma_c^2$ .

$e_{ijklmn}$  is the residual random error assumed with mean zero and variance  $\sigma_e^2$ .

The quadratic effect of deviation from herd median calving date was not included in the model because this effect was not significant. The fixed effect of feed cannot be included in the model because it is confounding with milking frequency. F-values were used to show the level of importance that the fixed effect had on the dependent variables. The F-value is obtained by dividing the mean sum of squares of the effect by the mean error sum of squares.

Monthly records of each cow on each milking frequency were used to model the BCS curves for each cow using a third-order polynomial.

#### **4.4. Results**

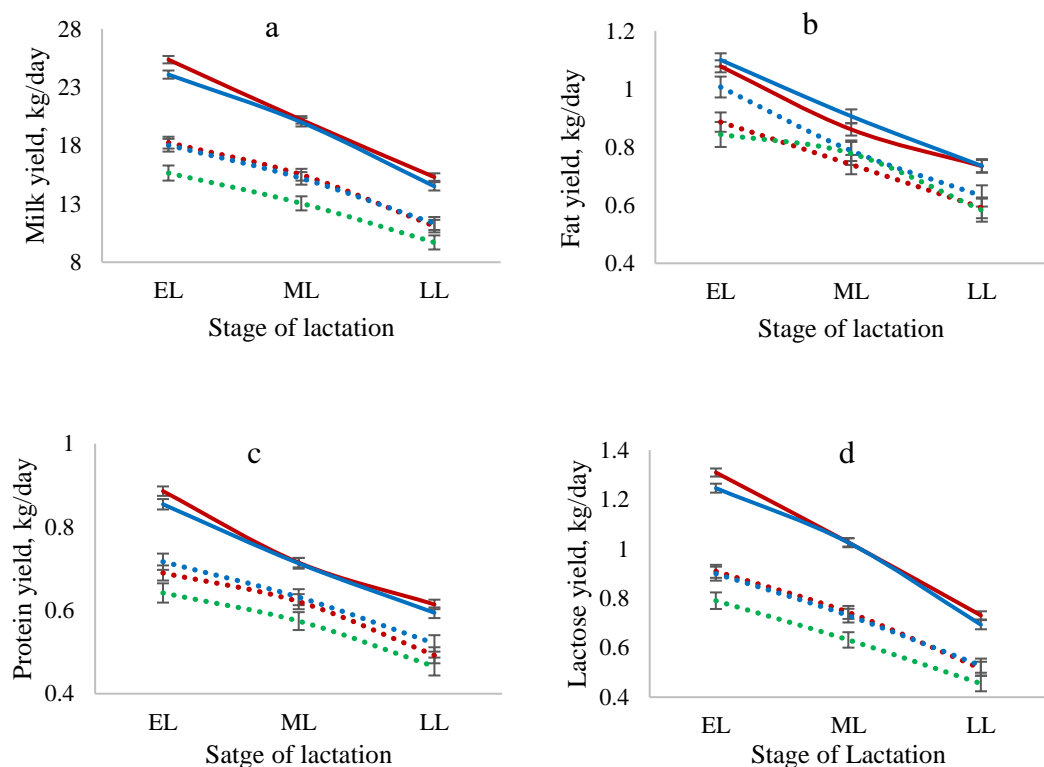
The means, standard deviations, F-values, and associated probabilities for each dependent variable from the analyses of variance are shown in Table 4.1. For most traits, the F statistics indicated that the stage of lactation, milking frequency, and lactation number explained the majority of the variation. Exceptions were proportions of C18:0 and LCFA, for which deviation from the median calving date was the most important independent variable. The effect of breed nested in milking frequency, the interaction effect of breed nested in milking frequency and stage of lactation, and the deviation from herd median calving date explained smaller, but significant amounts, of variation in the study variables.

**Table 4.1.** Means, standard deviations, and F-values for factors affecting production traits, milk composition, and fatty acid composition of cows milked once a day and twice a day during the 2020–2021 production season.

Variable	Mean	SD	F-values					
			Milking Frequency	Lactation Number	Breed (MF)	Stage of Lactation	Breed (MF)×S	dmcd
Milk yield, kg/day	18.1	5.8	320.5 ***	207.2 ***	6.7 ***	498.7 ***	6.9 ***	3.1 *
Fat yield, kg/day	0.84	0.3	58.7 ***	110.6 ***	2.4	153.8 ***	1.2 *	7.2 **
Protein yield, kg/day	0.67	0.2	146.6 ***	209.9 ***	3.6 *	288.9 ***	4.3 ***	0.7
Lactose yield, kg/day	0.88	0.32	372.7 ***	175.9 ***	5.2 **	586.3 ***	9.3 ***	3.5
Fat %	4.41	1.1	241.2 ***	2.2	15.8 ***	34.8 ***	8.6 ***	0.2
Protein %	3.92	0.4	236.4 ***	10.1 **	16.8 ***	851.6 ***	5.8 ***	7.8 **
Lactose %	4.95	0.3	127.3 ***	9.9 **	1.6	633.4 ***	11.8 ***	1.6
Fatty acid, % of the total FA								
SFA <sup>1</sup>	70.0	3.1	5.0 *	71.0 ***	4.6 **	215.4 ***	4.1 ***	3.7
UFA <sup>2</sup>	30.2	2.6	32.5 ***	87.5 ***	3.8 *	168.9 ***	5.1 ***	2.7
PUFA <sup>3</sup>	2.95	0.5	107.1 ***	64.0 ***	2.0	377.2 ***	73.8 ***	0.2
C4:0	4.01	0.3	167.7 ***	9.2 **	0.1	566.6 ***	28.4 ***	11.8 **
C6:0	2.81	0.2	13.4 ***	29.0 ***	1.0	435.8 ***	25.8 ***	1.1
C8:0	1.52	0.2	13.0 ***	28.6 ***	1.0	494.4 ***	23.3 ***	0.2
C10:0	3.42	0.4	45.4 ***	38.3 ***	1.0	283.0 ***	19.2 ***	1.0
C12:0	3.82	0.4	82.2 ***	32.7 ***	0.8	62.2 ***	10.8 ***	9.6 **
C14:0	12.7	1.3	5.3 *	45.3 ***	1.5	191.8 ***	4.9 ***	20.6 ***
C16:0	31.9	2.5	20.8 ***	51.8 ***	1.9	292.6 ***	3.6 ***	1.6
C18:0	13.1	1.5	8.2 **	5.1 *	9.6 ***	13.4 ***	10.1 ***	22.7 ***
C18:1 <i>cis</i> -9	20.2	2.8	8.0 **	60.6 ***	1.6	199.7 ***	7.1 ***	17.4 ***
Omega6	1.59	0.4	29.3 ***	19.7 ***	4.1 **	676.6 ***	65.5 ***	0.4
SCFA <sup>4</sup>	8.33	0.6	49.9 ***	24.2 ***	0.4	607.6 ***	32.0 ***	5.2 *
MCFA <sup>5</sup>	20.0	1.8	22.9 ***	47.1 ***	0.9	63.2 ***	6.4 ***	14.3 ***
LCFA <sup>6</sup>	66.7	2.6	63.5 ***	4.8 *	2.6	1.1	7.8 ***	30.0 ***

<sup>1</sup> SFA = Saturated fatty acids; <sup>2</sup> UFA = Unsaturated fatty acid; <sup>3</sup> PUFA = Polyunsaturated fatty acid. <sup>4</sup> SCFA = Short-chain fatty acids (sum of C4:0, C6:0 and C8:0); <sup>5</sup> MCFA = Medium-chain fatty acids (sum of C10:0, C12:0); <sup>6</sup> LCFA = Long-chain fatty acids (sum of C14:0, C16:0, C18:0, C18:1 *cis*-9 and Omega6). MF- milking frequency; S- stage of lactation; dmcd- deviation from median calving date. Statistical significance is given as: \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001.

Regardless of the significant ( $p < 0.05$ ) interaction between breeds within the milking frequency and stage of lactation, the daily yields of milk, fat, protein, and lactose declined from early to late lactation in both cows milked OAD and TAD and for all breeds (Figure 4.1). Cows milked TAD produced greater yields at all three stages of lactation. Jersey cows mostly produced significantly lower daily yields of milk, protein, and lactose compared with F and F×J, but similar levels of daily fat yield in mid-lactation and late lactation. The significant breed within the milking frequency and stage of lactation interaction most likely occurred in mid-lactation when between breed means changed in magnitude.

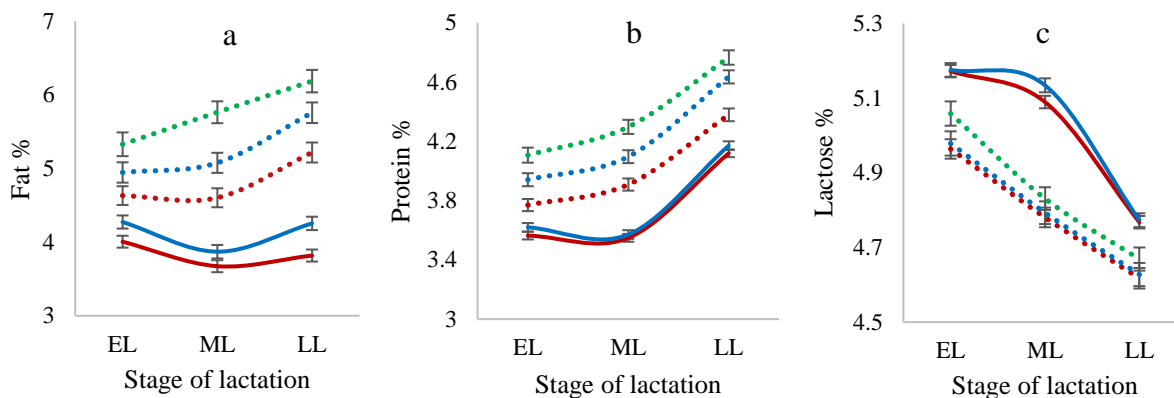


**Figure 4.1.** Daily yield of (a) milk, (b) fat, (c) protein, and (d) lactose in milk from Holstein-Friesian (....), Holstein-Friesian × Jersey (....) and Jersey (....) cows milked OAD, and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season.

EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

Figure 4.2 shows the percentages of fat, protein, and lactose in the three stages of lactation. In cows milked OAD, the percentages of fat and protein significantly ( $p < 0.05$ ) increased, and the percentages of lactose significantly decreased from early to late

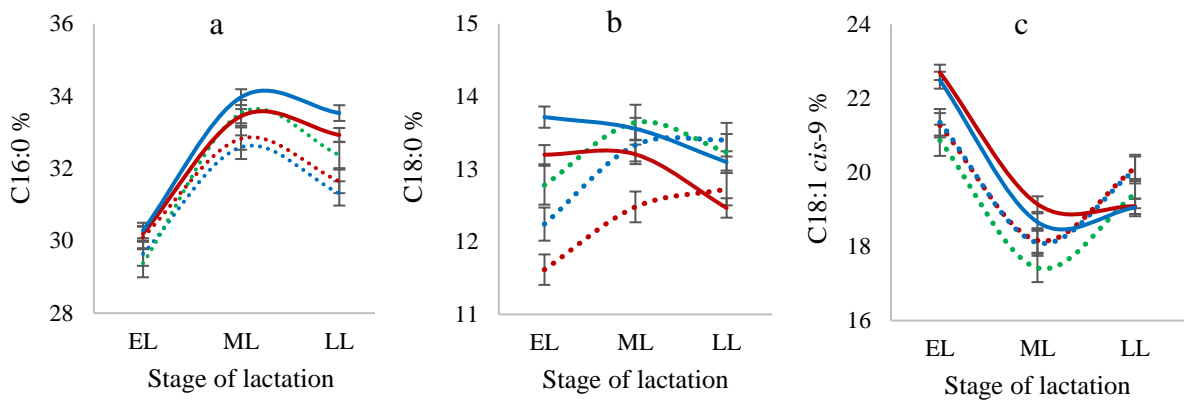
lactation. Jersey cows produced significantly higher percentages of fat and protein compared with the other two breeds. In cows milked TAD, F cows produced significantly higher fat percentages in early lactation compared with the other two stages of lactation whereas F×J cows produced significantly higher fat percentages in early and late lactation compared with mid-lactation. In early and late lactation, F×J cows produced significantly higher fat percentages compared with F cows.



**Figure 4.2.** Percentages of (a) fat, (b) protein, and (c) lactose in milk from Holstein-Friesian (....), Holstein-Friesian × Jersey (....), and Jersey (....) cows milked OAD, and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season.

EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

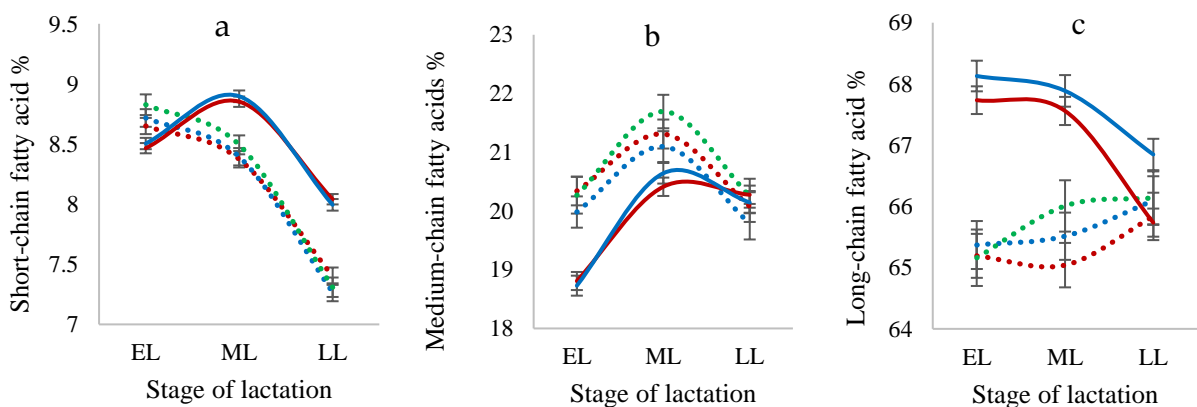
Figure 4.3 shows the proportions of C16:0, C18:0, and C18:1 *cis*-9 in the three stages of lactation. The proportion of C16:0 was significantly ( $p < 0.05$ ) higher in mid-lactation compared with early and late lactation, in cows milked OAD and TAD. In late lactation, J cows produced a significantly higher proportion of C16:0 compared with F×J cows milked OAD, whereas in the cows milked TAD, F×J cows produced a significantly higher proportion of C16:0 compared with F cows. Holstein-Friesian cows milked OAD, and TAD produced a significantly lower proportion of C18:0 throughout the lactation period and the proportion was significantly affected by stages of lactation. Holstein-Friesian × Jersey and J cows milked OAD did not show significant variation in the proportion of C18:0 in mid-lactation and late lactation. The proportion of C18:1 *cis*-9 was significantly higher in early lactation compared with mid- and late lactation in cows milked OAD and TAD, in all the breeds.



**Figure 4.3.** Proportion of (a) C16:0 (b) C18:0 (c) C18:1 *cis*-9 in milk from Holstein-Friesian (....), Holstein-Friesian × Jersey (.....), and Jersey (.....) cows milked OAD, and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season.

EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

The proportions of SCFA varied significantly ( $p < 0.05$ ) across the three stages of lactation in both milking frequencies (Figure 4.4). The proportions of MCFA were significantly higher in mid-lactation compared with early and late lactation in cows milked OAD. In late lactation, F×J cows produced significantly higher proportions of LCFA compared with F cows milked TAD.



**Figure 4.4.** Proportions of (a) short-chain fatty acids (b) medium-chain fatty acids (c) long-chain fatty acids in milk from Holstein-Friesian (....), Holstein-Friesian × Jersey, (.....) and Jersey (.....) cows milked OAD, and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020–2021 production season.

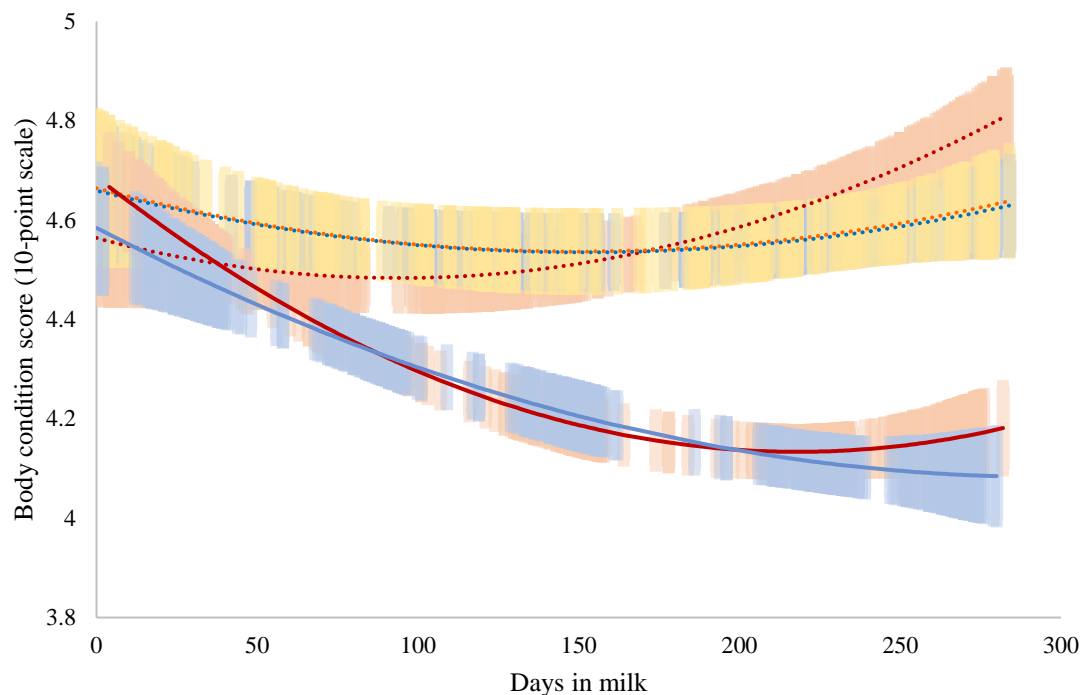
EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

The breed effect was significant for daily milk and fat yields, percentages of fat and protein, and proportions of SFA based on the F-values (Table 4.1). Least-square means and standard errors for each breed within milking frequency for milk production, composition traits, and fatty acid composition are presented in Table 4.2. In cows milked OAD, J cows showed a significantly ( $p < 0.05$ ) lower daily milk yield, protein yield, and lactose yield compared with F and F×J cows. Holstein-Friesian × Jersey cows produced a significantly higher daily fat yield. Jersey cows produced significantly higher percentages of fat and protein compared with F cows with F×J cows producing intermediate values. In cows milked TAD, F cows produced significantly higher daily milk yield, and F×J cows produced significantly higher fat and protein percentages. The breed effect for most of the fatty acids proportions was not significant in cows milked OAD and TAD. The proportions of SFA and C18:0 were significantly higher and the proportions of UFA were significantly lower in J cows milked OAD and F×J cows milked TAD. The results of this study have to be considered with caution due to the confounding effect of feed with milking frequency.

**Table 4.2.** Least-square means and standard errors for the production traits, milk composition, and fatty acid composition of milk from Holstein-Friesian (F), Holstein-Friesian × Jersey (F×J), and Jersey (J) cows milked once a day (OAD) and twice a day (TAD) (regardless of stages of lactation) in the 2020–2021 production season.

Variable	Milking Frequency				
	OAD			TAD	
	F	F × J	J	F	F × J
Milk yield, kg/day	15.34 ± 0.39 <sup>a</sup>	15.23 ± 0.40 <sup>a</sup>	13.18 ± 0.46 <sup>b</sup>	20.9 ± 0.25 <sup>a</sup>	20.12 ± 0.27 <sup>b</sup>
Fat yield, kg/day	0.74 ± 0.02 <sup>b</sup>	0.81 ± 0.02 <sup>a</sup>	0.74 ± 0.03 <sup>b</sup>	0.89 ± 0.01 <sup>a</sup>	0.91 ± 0.02 <sup>a</sup>
Protein yield, kg/day	0.6 ± 0.01 <sup>ab</sup>	0.62 ± 0.01 <sup>a</sup>	0.56 ± 0.02 <sup>b</sup>	0.74 ± 0.01 <sup>a</sup>	0.72 ± 0.01 <sup>a</sup>
Lactose yield, kg/day	0.72 ± 0.02 <sup>a</sup>	0.72 ± 0.02 <sup>a</sup>	0.63 ± 0.02 <sup>b</sup>	1.02 ± 0.01 <sup>a</sup>	0.99 ± 0.01 <sup>a</sup>
Fat %	4.82 ± 0.10 <sup>c</sup>	5.26 ± 0.11 <sup>b</sup>	5.76 ± 0.12 <sup>a</sup>	3.83 ± 0.06 <sup>b</sup>	4.13 ± 0.07 <sup>a</sup>
Protein %	4.02 ± 0.04 <sup>c</sup>	4.22 ± 0.04 <sup>b</sup>	4.39 ± 0.04 <sup>a</sup>	3.74 ± 0.02 <sup>a</sup>	3.79 ± 0.02 <sup>a</sup>
Lactose %	4.79 ± 0.02 <sup>a</sup>	4.8 ± 0.02 <sup>a</sup>	4.85 ± 0.03 <sup>a</sup>	5.01 ± 0.01 <sup>a</sup>	5.03 ± 0.02 <sup>a</sup>
Fatty acid, % of the total FA					
SFA <sup>1</sup>	69.47 ± 0.29 <sup>b</sup>	69.64 ± 0.3 <sup>b</sup>	70.73 ± 0.34 <sup>a</sup>	70.19 ± 0.18 <sup>b</sup>	70.76 ± 0.2 <sup>a</sup>
UFA <sup>2</sup>	30.93 ± 0.24 <sup>a</sup>	30.99 ± 0.25 <sup>a</sup>	30.14 ± 0.28 <sup>b</sup>	29.83 ± 0.15 <sup>a</sup>	29.35 ± 0.16 <sup>b</sup>
PUFA <sup>3</sup>	3.14 ± 0.03 <sup>a</sup>	3.09 ± 0.03 <sup>a</sup>	3.09 ± 0.04 <sup>a</sup>	2.86 ± 0.02 <sup>a</sup>	2.8 ± 0.02 <sup>b</sup>
C4:0	3.83 ± 0.03 <sup>a</sup>	3.84 ± 0.03 <sup>a</sup>	3.84 ± 0.03 <sup>a</sup>	4.11 ± 0.02 <sup>a</sup>	4.12 ± 0.02 <sup>a</sup>
C6:0	2.77 ± 0.02 <sup>a</sup>	2.76 ± 0.02 <sup>a</sup>	2.81 ± 0.02 <sup>a</sup>	2.83 ± 0.01 <sup>a</sup>	2.84 ± 0.01 <sup>a</sup>
C8:0	1.54 ± 0.01 <sup>a</sup>	1.53 ± 0.01 <sup>a</sup>	1.56 ± 0.02 <sup>a</sup>	1.51 ± 0.01 <sup>a</sup>	1.51 ± 0.01 <sup>a</sup>
C10:0	3.61 ± 0.04 <sup>a</sup>	3.54 ± 0.04 <sup>a</sup>	3.58 ± 0.05 <sup>a</sup>	3.38 ± 0.03 <sup>a</sup>	3.34 ± 0.03 <sup>a</sup>
C12:0	4.04 ± 0.04 <sup>a</sup>	3.97 ± 0.04 <sup>a</sup>	4.03 ± 0.04 <sup>a</sup>	3.76 ± 0.02 <sup>a</sup>	3.74 ± 0.03 <sup>a</sup>
C14:0	12.92 ± 0.12 <sup>ab</sup>	12.78 ± 0.12 <sup>a</sup>	13.14 ± 0.13 <sup>b</sup>	12.7 ± 0.07 <sup>a</sup>	12.76 ± 0.08 <sup>a</sup>
C16:0	31.52 ± 0.24 <sup>a</sup>	31.18 ± 0.26 <sup>a</sup>	31.76 ± 0.28 <sup>a</sup>	32.19 ± 0.15 <sup>a</sup>	32.59 ± 0.17 <sup>a</sup>
C18:0	12.27 ± 0.16 <sup>b</sup>	12.99 ± 0.17 <sup>a</sup>	13.22 ± 0.19 <sup>a</sup>	12.96 ± 0.1 <sup>b</sup>	13.46 ± 0.11 <sup>a</sup>
C18:1 <i>cis</i> -9	19.85 ± 0.24 <sup>a</sup>	19.84 ± 0.25 <sup>a</sup>	19.24 ± 0.28 <sup>a</sup>	20.31 ± 0.15 <sup>a</sup>	20.07 ± 0.16 <sup>a</sup>
Omega6	1.71 ± 0.03 <sup>a</sup>	1.63 ± 0.03 <sup>b</sup>	1.61 ± 0.03 <sup>b</sup>	1.55 ± 0.02 <sup>a</sup>	1.5 ± 0.02 <sup>b</sup>
SCFA <sup>4</sup>	8.14 ± 0.05 <sup>a</sup>	8.13 ± 0.05 <sup>a</sup>	8.21 ± 0.06 <sup>a</sup>	8.45 ± 0.03 <sup>a</sup>	8.47 ± 0.04 <sup>a</sup>
MCFA <sup>5</sup>	20.58 ± 0.18 <sup>a</sup>	20.29 ± 0.19 <sup>a</sup>	20.74 ± 0.21 <sup>a</sup>	19.83 ± 0.11 <sup>a</sup>	19.84 ± 0.12 <sup>a</sup>
LCFA <sup>6</sup>	65.36 ± 0.26 <sup>a</sup>	65.66 ± 0.29 <sup>a</sup>	65.78 ± 0.31 <sup>a</sup>	67.01 ± 0.17 <sup>b</sup>	67.62 ± 0.18 <sup>a</sup>

<sup>1</sup> SFA = Saturated fatty acids; <sup>2</sup> UFA = Unsaturated fatty acid; <sup>3</sup> PUFA = Polyunsaturated fatty acid. <sup>4</sup> SCFA = Short-chain fatty acids (sum of C4:0, C6:0 and C8:0); <sup>5</sup> MCFA = Medium-chain fatty acids (sum of C10:0, C12:0 and C14:0); <sup>6</sup> LCFA = Long-chain fatty acids (sum of C16:0, C18:0, C18:1 *cis*-9 and Omega6). <sup>a, b, c</sup> Means with different superscripts between breeds within milking frequency are significantly different ( $p < 0.05$ ).



**Figure 4.5.** Body condition score of Holstein-Friesian (...), Holstein-Friesian × Jersey (...), and Jersey (...) cows milked OAD and Holstein-Friesian (—), and Holstein-Friesian × Jersey (—) cows milked TAD on a 10-point scale during the 2020–2021 production season.

The shadings indicate 95% confidence intervals.

Figure 4.5 shows the BCS measured during the sampling period. The F×J and J cows milked OAD had a similar trend in BCS throughout the lactation period, which was higher than F cows in early and mid-lactation. The cows milked TAD showed lower BCS throughout lactation compared with the cows milked OAD.

#### 4.5. Discussion

The chemical composition of feed slightly differed between the farms (Table 4.1). The metabolisable energy contents of the feed provided for cows in both farms were similar (ranging between 10–12 ME MJ/kg DM). Under the grazing conditions of this study, the crude protein content of the feed was higher than the recommended level of 16–17 g/100g dry matter [31]. The neutral detergent fibre content of the feed given in No.4 Dairy was higher than the recommended level of 35% for cows grazing high-quality pasture [32].

In this study, OAD milking frequency consisted of F, F×J, and J cows but TAD milking frequency only consisted of F and F×J cows. Therefore, the breed was nested in milking frequency for statistical analysis. Milk fatty acid composition affects milk processability,

especially, butter hardness [11] and cheese coagulation parameters [9]. The lower proportions of MCFA, C16:0, C18:0, and the higher proportion of C18:1 *cis*-9 could make the butter softer in early lactation than in mid- and late lactation in F and F×J cows milked OAD. This is in agreement with Auldust et al. [15], who reported that the milk from early lactation had lower SCF<sub>10</sub> than mid- or late lactation milk. On the other hand, the higher proportions of MCFA, C16:0, C18:0, and lower proportion of C18:1 *cis*-9 from milk produced in mid-lactation would yield harder butter compared with the milk produced in early and late lactation from cows milked OAD. Cows milked TAD would also be expected to show similar characteristics for butter hardness as cows milked OAD, but the higher proportion of C18:0 in the early lactation would be likely to increase the hardness of the butter if the milk is processed.

In cows milked OAD, F milk would give softer butter in early and mid-lactation due to lower proportion of C18:0 compared with F×J and J milk as the proportions of other important fatty acids: C16:0 and C18:1 *cis*-9 that determine butter hardness [11] were not significantly different across the breeds. In late lactation, F and F×J cows produced significantly lower proportion of C16:0 compared with J cows, which could lead to softer butter hardness. Overall, this study showed F cows milked OAD would be more likely to produce softer butter in all three stages of lactation. Similarly, in cows milked TAD, in early lactation, F milk showed a lower proportion of C18:0, and in late lactation, lower proportions of C16:0 and C18:0 compared with F×J cows, which suggested that the butter produced from F milk would be softer than F×J cows in early and late lactation. In mid-lactation, these fatty acids were not significantly different across the breeds. It is also clear that the F cows milked OAD, and TAD could produce softer butter than F×J and J cows regardless of the milking frequencies.

Generally, F cows produced lower proportions of C18:0 compared with J cows, which suggests that butter produced from F cows is likely to be softer than the butter from J cows. Thus, this is consistent with MacGibbon [11] and Mackle et al. [33] who reported that SFC<sub>10</sub> was positively correlated with the proportions of high melting point long-chain saturated fatty acids and negatively correlated with the proportion of C18:1 *cis*-9. Generally, the proportions of C16:0 and C18:1 *cis*-9 are the key fatty acids that influence butter hardness [11,33] as they are the predominant fatty acids.

The rennet coagulation parameters are affected by the stage of lactation [34]. Lucey [35] reported that milk from late lactation was less suitable for cheese making because of a long clotting time and reduced curd firmness. The reasons proposed were higher milk pH and proteolysis of casein. Auld et al. [9] reported that the proportions of C8:0, C10:0, and C12:0 were positively correlated with curd firming time ( $K_{20}$ ) and positively correlated with curd firmness ( $A_{60}$ ), whereas the proportion of C18:1 *cis*-9 was negatively correlated with the curd firming time. However, Auld et al. [9] reported that the relationship between milk composition and coagulation parameters was not sufficiently reliable to make predictions about cheese quality. In the current study, higher proportions of C8:0, C10:0, and C12:0 was observed in early lactation in cows milked OAD and in mid-lactation in cows milked TAD (Figure 1 and Figure 2 of Appendix 2). It is not clear how individual fatty acids affect milk coagulation parameters. In addition, in this study, the breed did not affect the proportions of MCFAs and C18:1 *cis*-9 in all three stages of lactation.

In the current study, breed effects were significant for some traits. The higher percentages of fat and protein in J cows, lower percentages in F cows, and intermediate percentages in F×J cows concurred with Palladino et al. [14] and Lopez-Villalobos et al. [25]. Jersey cows produce milk with higher concentrations of total solids compared with milk produced by F cows [9]. The superiority of the J cows milked OAD for the percentages of fat and protein would be beneficial in the New Zealand payment scheme as Sneddon et al. [36] reported the J cows to have a greater milk value per litre of milk. The J cows milked OAD tend to produce significantly greater proportions of SFA than F cows, with F×J cows producing intermediate values. This is in agreement with Palladino et al. [14] and Lopez-Villalobos et al. [25]. The opposite pattern was apparent for the proportions of UFA, being the highest in the F cows. Similar results were reported by Soyeurt et al. [12] and Palladino et al. [14]. The proportions of long-chain saturated fatty acids were higher in J cows compared with F cows, as demonstrated by Auld et al. [9], who mentioned that the proportions of long-chain saturated fatty acids were higher and the proportions of long-chain unsaturated fatty acids were lower in J cows, compared with F cows, as was found in the present study. The higher proportion of C18:0 and lower proportion of C18:1 *cis*-9 in J cows may be due to lower level of conversion of C18:0 into C18:1 *cis*-9. DePeters et al. [37], Townsend et al. [38], and Drackley et al. [39] suggested that J cows have less active delta-9-desaturase, which converts C18:0 into

C18:1 *cis*-9. In this study, the breed effect on delta-9-desaturase was not tested for cows milked OAD and TAD. Holstein-Friesian cows produced a lower proportion of C18:0 compared with the J cows, which suggests that butter produced milk from F cows is likely to be softer than the butter from J cows, which is in agreement with MacGibbon [11] and Mackle et al. [33].

Milk proportions of C16:0, C18:0, C18:1 *cis*-9, and MCFA influence butter-making properties [11]. Short- and medium-chain FA are *de novo* synthesised in the mammary gland. This process is mostly driven by precursors and enzymes [40] and is influenced by the energy balance of the cows [13,41] and the feed. Milking frequency affects cow energy balance, with cows milked OAD having improved energy balance compared with cows milked TAD [42,43] (Figure 5). The limitation of this study was the lack of control over the feed. Therefore, further studies should be conducted to study the effect of the milking frequency with the control of the feed.

#### 4.6. Conclusions

Holstein-Friesians cows are more likely to produce softer butter compared with other breeds of cows regardless of the milking frequency due to the lower proportion of C18:0 in their milk. Holstein-Friesians cows milked OAD tend to produce softer butter in early and mid-lactation whereas they would be more likely to produce softer butter in early and late lactation when they were milked TAD compared with the other breed of cows. Jersey cows milked OAD and F×J cows milked TAD produced higher percentages of fat and protein and higher proportions of SFA. The relative proportions of C18:0 and C18:1 *cis*-9 suggested the breed effect on the activity of delta-9-desaturase was not significant, whereas the effect of stages of lactation could be significant. These results suggest that further studies should be undertaken to examine the effects of milking frequency and feeding levels, with the prospect that better decisions could be made when selecting breeds, individual cows, and feeding levels to improve milk processability.

#### References

1. Tong, M.J.; Clark, D.A.; Cooper, C.V. Once-a-day milking: Possible and profitable? *Proc. N. Z. Grassl. Assoc.* **2002**, *64*, 33–37. <https://doi.org/10.33584/jnzg.2002.64.2470>.

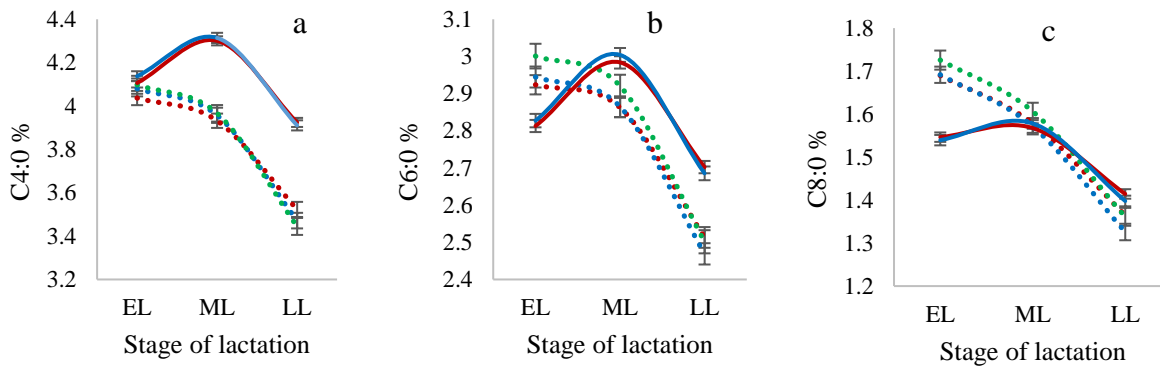
2. Davis, S.R. Lactational traits of importance in dairy cows and applications for emerging biotechnologies. *N. Z. Vet. J.* **2005**, *53*, 400–405. <https://doi.org/10.1080/00480169.2005.36584>.
3. Harris, B.L. Breeding dairy cows for the future in New Zealand. *N. Z. Vet. J.* **2005**, *53*, 384–390. <https://doi.org/10.1080/00480169.2005.36582>.
4. Livestock Improvement Corporation and DairyNZ. New Zealand Dairy Statistics 2019–20. 2020. Available online: [https://www.clal.it/upload/NZ\\_Dairy\\_Statistics\\_2019-20\\_WEB\\_FINAL.pdf](https://www.clal.it/upload/NZ_Dairy_Statistics_2019-20_WEB_FINAL.pdf) (accessed on 20 July 2021).
5. Lembeye, F.; López-Villalobos, N.; Burke, J.L.; Davis, S.R. Breed and heterosis effects for milk yield traits at different production levels, lactation number and milking frequencies. *N. Z. J. Agric. Res.* **2016**, *59*, 156–164. <https://doi.org/10.1080/00288233.2016.1156551>.
6. Dezetter, C.; Leclerc, H.; Mattalia, S.; Barbat, A.; Boichard, D.; Ducrocq, V. Inbreeding and crossbreeding parameters for production and fertility traits in Holstein, Montbéliarde, and Normande cows. *J. Dairy Sci.* **2015**, *98*, 4904–4913. <https://doi.org/10.3168/jds.2014-8386>.
7. Clasen, J.B.; Fogh, A.; Kargo, M. Differences between performance of F1 crossbreds and Holsteins at different production levels. *J. Dairy Sci.* **2019**, *102*, 436–441. <https://doi.org/10.3168/jds.2018-14975>.
8. Clasen, J.B.; Fikse, W.F.; Kargo, M.; Rydhmer, L.; Strandberg, E.; Østergaard, S. Economic consequences of dairy crossbreeding in conventional and organic herds in Sweden. *J. Dairy Sci.* **2020**, *103*, 514–528. <https://doi.org/10.3168/jds.2019-16958>.
9. Auldist, M.J.; Johnston, K.A.; White, N.J.; Fitzsimons, W.P.; Boland, M.J. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *J. Dairy Sci.* **2004**, *71*, 51–57. <https://doi.org/10.1017/s0022029903006575>.
10. MacGibbon, A.K.H.; Taylor, M.M. Composition and structure of bovine milk lipids. In *Advanced Dairy Chemistry*, 3rd ed.; Fox, P.F., McSweeney, P.L.H., Eds.; Springer: New York, NY, USA, 2006; Volume 2, pp. 1–42.
11. MacGibbon, A.K. Herd-to-herd variations in the properties of milkfat. *Proc. N. Z. Soc. Anim. Prod.* **1996**, *56*, 224–227.
12. Soyeurt, H.; Dardenne, P.; Gillon, A.; Croquet, C.; Vanderick, S.; Mayeres, P.; Bertozzi, C.; Gengler, N. Variation in fatty acid contents of milk and milk fat within

- and across breeds. *J. Dairy Sci.* **2006**, *89*, 4858–4865. [https://doi.org/10.3168/jds.S0022-0302\(06\)72534-6](https://doi.org/10.3168/jds.S0022-0302(06)72534-6).
13. Stoop, W.M.; Bovenhuis, H.; Heck, J.M.L.; van Arendonk, J.A.M. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *J. Dairy Sci.* **2009**, *92*, 1469–1478. <https://doi.org/10.3168/jds.2008-1468>.
  14. Palladino, R.A.; Buckley, F.; Prendiville, R.; Murphy, J.J.; Callan, J.; Kenny, D.A. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. *J. Dairy Sci.* **2010**, *93*, 2176–2184. <https://doi.org/10.3168/jds.2009-2453>.
  15. Auldist, M.J.; Walsh, B.J.; Thomson, N.A. Seasonal and lactational influences on bovine milk composition in New Zealand. *J. Dairy Res.* **1998**, *65*, 401–411. <https://doi.org/10.1017/s0022029998002970>.
  16. Back, P.J.; Thomson, N.A. Exploiting cow genotype to increase milk value through production of minor milk components. *Proc. N. Z. Soc. Anim. Prod.* **2005**, *65*, 53–58.
  17. Palmquist, D.L.; Beaulieu, A.D.; Barbano, D.M. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* **1993**, *76*, 1753–1771. [https://doi.org/10.3168/jds.S0022-0302\(93\)77508-6](https://doi.org/10.3168/jds.S0022-0302(93)77508-6).
  18. Dewhurst, R.J.; Shingfield, K.J.; Lee, M.R.; Scollan, N.D. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Feed Sci. Technol.* **2006**, *131*, 168–206. <https://doi.org/10.1016/j.anifeedsci.2006.04.016>.
  19. Heck, J.M.; Van Valenberg, H.J.; Dijkstra, J.; Van Hooijdonk, A.C. Seasonal variation in the Dutch bovine raw milk composition. *J. Dairy Sci.* **2009**, *92*, 4745–4755. <https://doi.org/10.3168/jds.2009-2146>.
  20. Schwendel, B.H.; Morel, P.C.; Wester, T.J.; Tavendale, M.H.; Deadman, C.; Fong, B.; Shadbolt, N.M.; Thatcher, A.; Otter, D.E. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. *J. Dairy Sci.* **2015**, *98*, 1411–1425. <https://doi.org/10.3168/jds.2014-8322>.
  21. Yang, W.; Zhang, B.; Xu, C.; Zhang, H.; Xia, C. Effects of ketosis in dairy cows on blood biochemical parameters, milk yield and composition, and digestive capacity. *J. Vet. Res.* **2019**, *63*, 555–560. <https://doi.org/10.2478/jvetres-2019-0059>.
  22. Guliński, P. Ketone bodies—causes and effects of their increased presence in cows' body fluids: A review. *Vet. World* **2021**, *14*, 1492–1503. <https://doi.org/10.14202/vetworld.2021.1492-1503>.

23. Xu, C.; Shen, T.; Yang, W.; Yu, H.; Gao, S.; Huang, B. The effect of subacute ruminal acidosis of dairy cows on productivity, digestibility and greenhouse gas emission. *J. Agric. Sci.* **2016**, *8*, 92–100. <https://doi.org/10.5539/jas.v8n4p92>.
24. Nejash, A. Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: A review of past and recent research at global prospective. *Achieve. Life Sci.* **2016**, *10*, 187–196. <https://doi.org/10.1016/j.als.2016.11.006>.
25. Lopez-Villalobos, N.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Holroyd, S.E.; MacGibbon, A.K.; Snell, R.G. Estimation of genetic and crossbreeding parameters of fatty acid concentrations in milk fat predicted by mid-infrared spectroscopy in New Zealand dairy cattle. *J. Dairy Sci.* **2014**, *81*, 340–349. <https://doi.org/10.1017/S0022029914000272>.
26. Arnould, V.R.; Soyeurt, H. Genetic variability of milk fatty acids. *J. Appl. Genet.* **2009**, *50*, 29–39. <https://doi.org/10.1007/BF03195649>.
27. Lembeye, F.; López-Villalobos, N.; Burke, J.L.; Davis, S.R. Estimation of breed and heterosis effects for milk traits and somatic cell scores in cows milked once and twice daily in New Zealand. *Proc. N. Z. Soc. Anim. Prod.* **2015**, *75*, 60–63.
28. Kgwatalala, P.M.; Ibeagha-Awemu, E.M.; Mustafa, A.F.; Zhao, X. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. *Anim. Genet.* **2009**, *40*, 609–615. <https://doi.org/10.1111/j.1365-2052.2009.01887.x>.
29. Bilal, G.; Cue, R.I.; Mustafa, A.F.; Hayes, J.F. Effects of parity, age at calving and stage of lactation on fatty acid composition of milk in Canadian Holsteins. *Can. J. Anim. Sci.* **2014**, *94*, 401–410. <https://doi.org/10.4141/cjas2013-172>.
30. DairyNZ. Body Condition Scoring Made Easy. 2004. Available online: <https://www.dairynz.co.nz/media/5790783/body-condition-scoring-made-easy-booklet.pdf> (accessed on 16 September 2021).
31. Alderman, G.; Cottrill, B.R. *Energy and Protein Requirements of Ruminants: An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients*; CAB International: Wallingford, UK, 1993; pp. 62–63.
32. DairyNZ. Dietary Fibre is Needed by the Cow to Maintain Healthy Rumen Function. Eating Fibre Stimulates Chewing, Saliva Production and Rumination. Available online: <https://www.dairynz.co.nz/feed/nutrition/principles-of-nutrition/fibre/> (accessed on 15 July 2022).

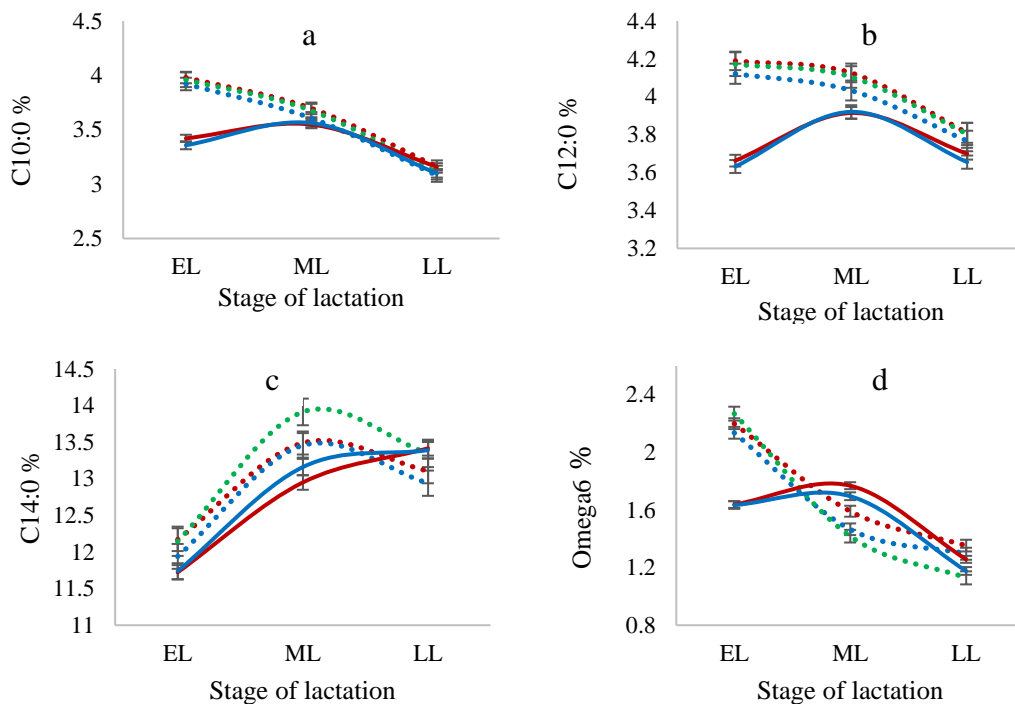
33. Mackle, T.R.; Petch, S.F.; Bryant, A.M.; Auld, M.J.; Henderson, H.V.; MacGibbon, A.K.H. Variation in the characteristics of milkfat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *N. Z. J. Agric. Res.* **1997**, *40*, 349–359. <https://doi.org/10.1080/00288233.1997.9513254>.
34. Lucey, J.A.; Fox, P.F. Rennet coagulation properties of late-lactation milk, Effect of pH adjustment, addition of CaCl<sub>2</sub>, variation in rennet level and blending with mid-lactation milk. *Ir. J. Agric. Food Res.* **1992**, *31*, 173–184.
35. Lucey, J. Cheesemaking from grass based seasonal milk and problems associated with late-lactation milk. *Int. J. Dairy Technol.* **1996**, *49*, 59–64. <https://doi.org/10.1111/j.1471-0307.1996.tb02491.x>.
36. Sneddon, N.W.; Lopez-Villalobos, N.; Hickson, R.E.; Shalloo, L.; Garrick, D.J. Estimation of crossbreeding effects on yields of dairy products and value of milk processed in different product portfolios. *Proc. N. Z. Soc. Anim. Prod.* **2015**, *75*, 48–53.
37. DePeters, E.J.; Medrano, J.F.; Reed, B.A. Fatty acid composition of milk fat from three breeds of dairy cattle. *Can. J. Anim. Sci.* **1995**, *75*, 267–269.
38. Townsend, S.J.; Siebert, B.D.; Pitchford, W.S. Variation in milk fat content and fatty acid composition of Jersey and Friesian cattle. *Proc. Assoc. Adv. Anim. Breed. Genet.* **1997**, *12*, 283–291.
39. Drackley, J.K.; Beaulieu, A.D.; Elliott, J.P. Responses of milk fat composition to dietary fat or nonstructural carbohydrates in Holstein and Jersey cows. *J. Dairy Sci.* **2001**, *84*, 1231–1237. [https://doi.org/10.3168/jds.S0022-0302\(01\)74584-5](https://doi.org/10.3168/jds.S0022-0302(01)74584-5).
40. MacGibbon, A.K.H. Composition and Structure of Bovine Milk Lipids. In *Advanced Dairy Chemistry*, 4th ed.; McSweeney, P.L.H., Fox, P.F., O'Mahony, J.A., Eds.; Springer: New York, NY, USA, 2020; Volume 2, pp. 1–32.
41. Gross, J.; van Dorland, H.A.; Bruckmaier, R.M.; Schwarz, F.J. Milk fatty acid profile related to energy balance in dairy cows. *J. Dairy Sci.* **2011**, *78*, 479–488.
42. Rémond, B.; Coulon, J.B.; Nicloux, M.; Levieux, D. Effect of temporary once-daily milking in early lactation on milk production and nutritional status of dairy cows. *Ann. Zootech.* **1999**, *48*, 341–352. <https://hal.archives-ouvertes.fr/hal-00889807/document>
43. Rémond, B.; Aubailly, S.; Chilliard, Y.; Dupont, D.; Pomiès, D.; Petit, M. Combined effects of once-daily milking and feeding level in the first three weeks of lactation on milk production and enzyme activities, and nutritional status, in Holstein cows. *Anim. Res.* **2002**, *51*, 101–117. <https://doi.org/10.1051/animres:2002014>.

### Appendix 2 (Supplementary information for Chapter 4)



**Appendix 2 Figure 1.** Proportions of (a) C4:0, (b) C6:0 and (c) C8:0 in milk from Holstein-Friesian (....), Holstein-Friesian × Jersey (....), and Jersey (....) cows milked OAD and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020-2021 production season.

EL = early lactation (<90 days); ML = mid lactation (90-180 days); LL= late lactation (>180 days). The vertical bars show standard errors.



**Appendix 2 Figure 2.** Proportions of (a) C10:0, (b) C12:0, (c) C14:0, and (d) omega6 in milk from Holstein-Friesian (....), Holstein-Friesian × Jersey (....), and Jersey (....) cows milked OAD and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020-2021 production season.

EL = early lactation (<90 days); ML = mid lactation (90-180 days); LL= late lactation (>180 days). The vertical bars show standard errors.

## **Foreword to Chapter 5**

This chapter studied the association between single nucleotide polymorphism rs109421300 and the fatty acid composition. All the cows from the No. 1 Dairy farm (once-a-day milking) and the young mob containing first and second parity Holstein-Friesian and Holstein-Friesian × Jersey cow from the No. 4 Dairy farm (twice-a-day milking) were selected.



## Chapter 5

### Association of single nucleotide polymorphism in the DGAT1 gene with the fatty acid composition of cows milked once and twice a day

Published as:

Sanjayanj, I.; MacGibbon, A.K.H.; Holroyd, S.E.; Janssen, P.W.M.; Blair, H.T.; Lopez-Villalobos, N. Association of single nucleotide polymorphism in the DGAT1 gene with the fatty acid composition of cows milked once and twice a day. *Genes*. 2023, *14*, 767.

<https://doi.org/10.3390/genes14030767>



## 5.1. Abstract

A single nucleotide polymorphism (SNP) rs109421300 of the diacylglycerol acyltransferase 1 (DGAT1) on bovine chromosome 14 is associated with fat yield, fat percentage, and protein percentage. This study aimed to investigate the effect of SNP rs109421300 on production traits and the fatty acid composition of milk from cows milked once a day (OAD) and twice a day (TAD) under New Zealand grazing conditions. Between September 2020 and March 2021, 232 cows from a OAD herd and 182 cows from a TAD herd were genotyped. The CC genotype of SNP rs109421300 was associated with significantly ( $p < 0.05$ ) higher fat yield, fat percentage, and protein percentage, and lower milk and protein yields in both milking frequencies. The CC genotype was also associated with significantly ( $p < 0.05$ ) higher proportions of C16:0 and C18:0, higher predicted solid fat content at 10°C (SFC<sub>10</sub>), and lower proportions of C4:0 and C18:1 *cis*-9 in both milking frequencies. The association of SNP rs109421300 with fatty acids was similar in both milking frequencies, with differences in magnitudes. The SFC<sub>10</sub> of cows milked OAD was lower than cows milked TAD for all three SNP genotypes suggesting the suitability of OAD milk for producing easily spreadable butter. These results demonstrate that selecting cows with the CC genotype is beneficial for New Zealand dairy farmers with the current payment system, however, this would likely result in less spreadable butter.

Keywords: dairy cattle; DGAT1; fatty acids; milking frequency; SNP genotype

## 5.2. Introduction

In New Zealand, twice-a-day (TAD) milking is the standard milking method of milk harvest. However, in the late 1990s farmers started to adopt once-a-day (OAD) milking due to several benefits such as reduced labour cost, improved labour efficiency, improved health and reproductive performance of cows, and additional employment opportunities for farmers [1–3]. Once-a-day milking tends to reduce the milk yield and increase the percentages of fat and protein compared with TAD milking [2,4,5].

When considering milk composition, fat is an important component that plays an important role in the New Zealand milk payment scheme [6] and the processability of milk [7]. Bovine milk fat is made up of 98% triglycerides [8]. Triglycerides consist of glycerol and three fatty acids and are mainly synthesised from diglycerides and this step is catalysed by diacylglycerol acyltransferase enzymes (DGAT1 and DGAT2). Fatty

acids in milk are derived from two main sources: the short- and medium-chain fatty acids are produced by *de novo* synthesis and long-chain fatty acids are absorbed directly from the diet [9]. Fatty acid composition is also affected by breed [10–12], stage of lactation [13,14], diet [15,16], and season [17,18]. Another important factor influencing the milk fatty acid composition within the breed is genetic variation. The fatty acid composition of milk is also considered important in influencing the processability of milk, especially butter making. The fatty acid composition of the milk influences the butter hardness by affecting the solid fat content at 10°C (SFC<sub>10</sub>) [19]. Butter hardness reflects the ease with which the butter can be spread on bread [20].

Recently, one of the dairy industry's main interests is to understand cows' genetic variation for desired traits to make a better selection process. The fatty acid composition of cow milk is one trait that is widely studied [10,21,22]. Several studies have reported the effect of the diacylglycerol acyltransferase 1 (DGAT1) polymorphism in the bovine genome on major milk traits of cows [23–25]. Some studies have also reported the effect of DGAT1 polymorphism on the fatty acid composition of milk [25–27]. However, there are some other polymorphisms in proximity to DGAT1 genes that could contribute to the variation in the fatty acid composition of cow milk. The quantitative trait locus (QTL) which has a major effect on milk production traits is positioned at the centromere end of chromosome 14 where the DGAT1 gene is located. The association of the single nucleotide polymorphism (SNP; rs109421300) of DGAT1 with fat and protein percentages in cows milked once a day (OAD) and twice a day (TAD) has been reported by Lopez-Villalobos et al. [28]. The authors reported that CC genotype of the SNP was associated with higher percentages of fat and protein compared with TT genotypes. However, the effect of this SNP on the milk fatty acid composition has not been tested yet. This study aimed to investigate the effect of SNP rs109421300 on the fatty acid composition of cows milked OAD and TAD.

### **5.3. Materials and Methods**

#### **5.3.1. Farm and feeding**

Cows on the Massey University No.1 Dairy farm are fed ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture with a low level of supplementation and were milked OAD in the mornings. The stocking rate of the No. 1 Dairy is 2.4 cows/ha. Cows on the Massey University No. 4 Dairy farm are fed ryegrass and white clover pasture with

a high level of supplementation and were milked TAD in the mornings and afternoons. The stocking rate of the No. 4 Dairy is 2.6 cows/ha. The feed allocation and the chemical composition of feed given to cows on both farms in the 2020–2021 production season are presented in Table 5.1.

**Table 5.1.** Feed allocation and chemical composition of diet provided at the No. 1 Dairy and No. 4 Dairy during the sampling period in the 2020–2021 production season.

Farm	No. 1 Dairy (OAD <sup>13</sup> )	No. 4 Dairy (TAD <sup>14</sup> )
Feed allocation (kg DM <sup>1</sup> per cow per day)		
Pasture <sup>2</sup>	8.2	14.7
Herb mix crop <sup>3</sup>	3.7	-
Maize silage	1.0	4.0
DDG <sup>4</sup>	1.5	1.0
Tapioca pellets	0.8	-
Concentrate <sup>5</sup>	2.0	2.5
Dry roughage <sup>6</sup>	-	0.6
Baleage <sup>7</sup>	4.0	5.5
Feed chemical composition		
ME <sup>8</sup> MJ/kg DM	11.33	11.07
CP <sup>9</sup> g/100 g DM	20.49	19.95
NDF <sup>10</sup> g/100 g DM	38.91	44.43
ADF <sup>11</sup> g/100 g DM	22.44	23.24
SSS <sup>12</sup> g/100 g DM	13.22	15.09
Lipid g/100 g DM	4.44	4.42

<sup>1</sup> DM = dry matter; <sup>2</sup> Comprised perennial ryegrass (L.p) and white clover (T.r); <sup>3</sup> comprised plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), and red clover (*Trifolium pratense*); <sup>4</sup> dried distillers grains; <sup>5</sup> grain-based concentrate; <sup>6</sup> hay and straw; <sup>7</sup> Lucerne baleage in No. 1 Dairy and grass baleage in No. 4 Dairy; <sup>8</sup> metabolisable energy; <sup>9</sup> crude protein; <sup>10</sup> neutral detergent fibre; <sup>11</sup> acid detergent fibre; <sup>12</sup> SSS = soluble sugars and starch; <sup>13</sup> once-a-day milking; <sup>14</sup> twice-a-day milking.

### 5.3.2. Cows and milk sampling

A herd from No. 1 Dairy (OAD milking herd), comprised 232 cows with a breed proportion of 70 Holstein-Friesian (F), 104 Holstein-Friesian × Jersey (F×J), and 58 Jersey (J) cows, and another herd from No. 4 Dairy (TAD milking herd) comprised of 182 cows with the breed proportion of 102 F and 80 F×J cows were selected for this study. The two herds contained primiparous (No. 1 Dairy = 55 and No. 4 Dairy = 118) and multiparous (No. 1 Dairy = 177 and No. 4 Dairy = 64) cows. The cows from both herds were free from clinical mastitis and metabolic diseases during the sampling period. The milk samples were collected during early (September 2020), mid- (December 2020), and

late (March 2021) lactation in the 2020–2021 production season. The cows had a minimum of three records for the production season. Milk samples were collected using Waikato milk flow meters. The samples were stored in the refrigerator at 0–4°C immediately after collection without adding any preservatives and were analysed for milk composition within two days of collection.

### 5.3.3. *Determination of milk composition and fatty acid composition*

Using standard calibration equations, all samples were analysed for fat, protein, and lactose percentages by a Milkoscan FT1 (Foss, Hillerød, Denmark). Calibration equations for individual fatty acids were developed using the FTIR calibrator software (Foss Analytical, Hillerød, Denmark) with the reference values obtained from gas chromatography (GC) [29] with Shimadzu GC-2010 plus. The proportions of individual fatty acids were predicted using the calibration equations in Milkoscan FT1 (Foss, Hillerød, Denmark).

### 5.3.4. *Genotyping*

DNA extracted from ear punch tissue samples were genotyped using Bovine Illumina 50K SNP chips. The analysis used all the animals that met the call rate of 80%. The SNP rs109421300 was selected for the statistical analysis because of the proximity to the DGAT1 gene and the significant effect on fat percentage.

### 5.3.5. *Statistical analysis*

Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Least-square means and standard errors for the study traits were obtained using the PROC MIXED procedure with the following linear mixed model:

$$Y_{ijklmn} = \mu + M_i + B_j(M_i) + L_k + S_l + GM_{mi} + \beta_1 d_n + C_n + e_{ijklmn}$$

where

$Y_{ijklmn}$  is the observation for any of the production and composition traits in milking frequency  $i$ , breed  $j$ , lactation number  $k$ , stage of lactation  $l$ , genotypes  $m$ , and cow  $n$ .

$\mu$  is the population mean.

$M_i$  is the fixed effect of milking frequency ( $i = \text{OAD and TAD}$ ).

$B_j(M_i)$  is the fixed effect of breed  $j$  nested in milking frequency  $i$  ( $j = \text{F, F} \times \text{J, and J}$ ).

$L_k$  is the fixed effect of lactation number ( $k = 1, 2, \dots, 5$ ).

Sl is the fixed effect of the stage of lactation (l = early, mid, and late).

GMmi is the fixed effect of interaction between genotype m and milking frequency i.

$\beta_1$  is the regression coefficient of the linear effect of deviation (dn; days) from herd median calving date on trait Y of cow n.

Cn is the random effect of cow (n = 1,2, ..., 414) assumed with mean zero and variance  $\sigma_c^2$ .

eijklmn is the residual random error assumed with mean zero and variance  $\sigma_e^2$ .

A limitation of this study was the confounding effect of feed and milking frequency. The feed provided for cows on each farm was slightly different. Cows milked TAD were provided more supplements compared with cows milked OAD. The genetic merit of the cows on both farms was similar as they were from the same few sires. The breed was nested in milking frequency as the two milking frequencies had different breed proportions: F, F×J, and J cows in OAD milking and F and F×J in TAD milking.

Solid fat content at 10°C was predicted from an equation developed using PROC REG. The prediction equation was developed using the fatty acid composition and SFC<sub>10</sub> data from the study of MacGibbon [19]. Using the same PROC MIXED model, the effects of SNP genotypes on fatty acids C4:0, C16:0, and C18:1 *cis*-9, and SFC<sub>10</sub> at different stages of lactation in cows milked OAD and TAD were estimated by including the interaction between SNP genotypes, milking frequency and stages of lactation as a fixed effect. Partial correlation coefficients (adjusted by the factors in the model) between the study traits were obtained using MANOVA. The SNP variances for the traits were obtained by fitting the SNP rs109421300 as a random effect in the model.

#### 5.4. Results

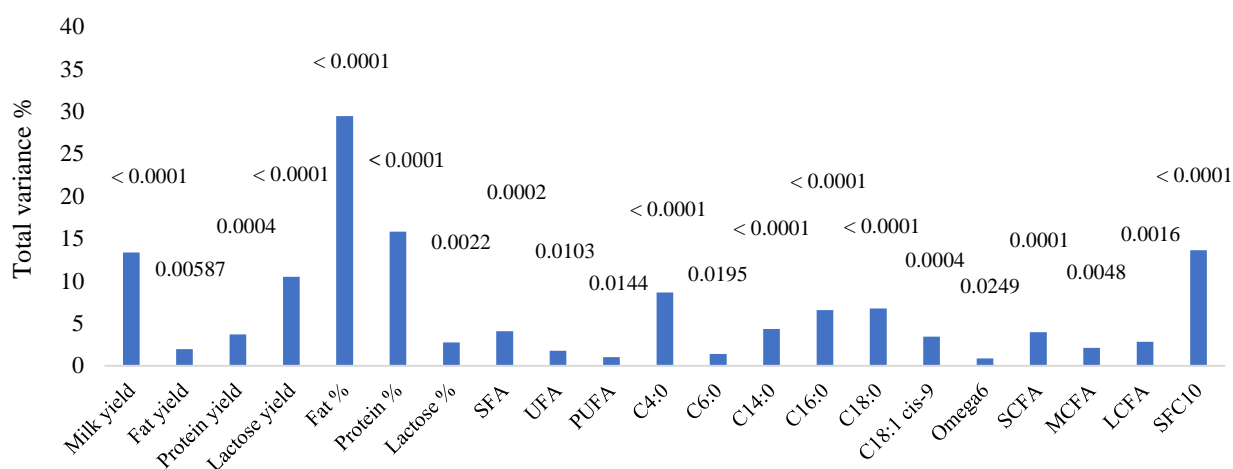
The frequency of genotypes CC, CT, and TT were 31.9, 44.2, and 23.9%, respectively, in the OAD herd, and 19.8, 52.1, and 28.1%, respectively, in the TAD herd. The frequency of the C and T alleles were 54 and 46%, respectively, in the OAD herd while the frequencies were 46 and 54% in the TAD herd, respectively. The p-values for the chi-square test for the OAD herd and TAD herd were 0.1001 and 0.524, respectively. The p-values ( $p > 0.05$ ) from the Chi-square test suggest that the population was at equilibrium.

**Table 5.2.** Descriptive statistics of production traits, gross milk composition, and fatty acid composition of cows milked once a day (OAD) and twice a day (TAD) during the 2020–2021 production season.

Milking frequency	OAD						TAD					
	Variable	N	Mean	SD	CV%	Min	Max	N	Mean	SD	CV%	Min
Milk yield, kg/day	668	18.43	6.3	35.2	4.73	41.1	543	20.1	5.56	28.5	6.6	40.69
Fat yield, kg/day	668	0.91	0.32	35.4	0.26	5.22	543	0.88	0.22	24.6	0.31	1.92
Protein yield, kg/day	668	0.73	0.22	30.3	0.21	1.48	543	0.71	0.17	23.6	0.29	1.54
Lactose yield, kg/day	668	0.87	0.33	38	0.19	2.13	543	0.99	0.30	30.5	0.29	2.08
Fat %	694	4.86	1.04	21.3	1.72	8.53	543	3.98	0.85	21.4	1.27	6.89
Protein %	694	4.14	0.4	9.6	3.19	5.67	543	3.76	0.37	9.9	2.74	5.01
Lactose %	694	4.83	0.26	5.3	3.7	5.43	543	5.03	0.24	4.7	4.29	5.51
Fatty acid, % of the total FA												
SFA <sup>1</sup>	694	70.65	3.31	4.7	51.57	81.78	543	70.08	2.96	4.2	58.64	77.94
UFA <sup>2</sup>	694	29.85	2.68	9	21.71	43.96	543	29.94	2.54	8.5	23.70	38.84
PUFA <sup>3</sup>	694	3.13	0.52	16.7	2.01	4.61	543	2.87	0.41	14.5	1.49	3.85
C4:0	694	3.95	0.34	8.7	2.5	4.89	543	4.11	0.25	6.0	2.52	4.65
C6:0	694	2.87	0.25	8.7	1.37	3.49	543	2.83	0.20	6.9	1.71	3.29
C8:0	694	1.6	0.17	10.7	0.56	2.05	543	1.50	0.12	8.3	0.68	1.82
C10:0	694	3.7	0.46	12.3	0.93	4.93	543	3.33	0.34	10.3	1.19	4.38
C12:0	694	4.08	0.37	9.1	1.9	5.17	543	3.72	0.29	7.7	2.46	4.46
C14:0	693	13.07	1.35	10.3	5.44	16.05	543	12.58	1.22	9.7	7.14	15.27
C16:0	694	31.52	2.83	9	22.86	38.69	543	32.10	2.47	7.7	25.17	39.81
C18:0	694	12.62	1.59	12.6	7.87	22.23	543	13.28	1.22	9.2	9.94	21.37
C18:1 <i>cis</i> -9	694	19.18	2.76	14.4	11.81	37.88	543	20.54	2.69	13.1	14.75	31.99
Omega-6	694	1.74	0.51	29.5	0.47	4.03	543	1.55	0.31	20.1	0.37	2.28
SCFA <sup>4</sup>	694	8.41	0.72	8.6	4.43	10.02	543	8.44	0.53	6.3	5.37	9.71
MCFA <sup>5</sup>	694	20.83	1.91	9.2	8.64	25.43	543	19.63	1.64	8.4	11.57	23.37
LCFA <sup>6</sup>	694	65.06	2.38	3.7	58.25	79.77	543	67.46	2.41	3.6	62.12	82.02
Milk income, NZD/day <sup>7</sup>	668	14.8	4.64	31.3	4.26	57.33	543	14.34	3.37	23.5	6.27	31.16
SFC <sub>10</sub> <sup>8</sup>	694	67.53	8.6	12.7	17.7	89.57	543	67.44	7.13	10.6	41.03	86.96

<sup>1</sup> Saturated fatty acids; <sup>2</sup> Unsaturated fatty acids; <sup>3</sup> Polyunsaturated fatty acids; <sup>4</sup> Short-chain fatty acids (sum of C4:0, C6:0, and C8:0); <sup>5</sup> Medium-chain fatty acids (sum of C10:0, C12:0, C14:0); <sup>6</sup> Long-chain fatty acids (sum of C16:0, C18:0, C18:1 *cis*-9 and omega-6); <sup>7</sup> Milk income was calculated using the equation NZD 9.78 × kg fat yield + NZD 8.89 × kg protein yield – NZD 0.033 × kg milk yield; <sup>8</sup> Solid fat content at 10°C; N is from 3 sampling periods (early, mid, and late lactation).

The descriptive statistics for the production traits and composition traits of cows milked OAD and TAD are presented in Table 5.2. Figure 5.1 shows the proportion of variance explained by the selected SNP rs109421300 and the probability values for the variance. The SNP explained approximately 30% of the variance for fat percentage, 15% for protein percentage, and 13% for milk yield. For fatty acids, approximately 10% of the variance for C4:0 was explained by the SNP and 6% for C16:0 and C18:0. The SNP did not control a significant proportion of variance for fatty acids C8:0, C10:0, and C12:0. Approximately 14% of the variance was explained by the SNP for the variable SFC<sub>10</sub>.



**Figure 5.1.** The proportions of variance explained by the SNP rs109421300 for the production traits and composition traits in cows milked in the 2020–2021 production season.

SFA = saturated fatty acids; UFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids; SCFA = short-chain fatty acids (sum of C4:0, C6:0, and C8:0); MCFA = medium-chain fatty acids (sum of C10:0, C12:0); LCFA = long-chain fatty acids (sum of C14:0, C16:0, C18:0, C18:1 *cis*-9, and omega-6); SFC<sub>10</sub> = Solid fat content at 10 °C.

The partial correlation coefficients between fat percentage and fatty acid composition of milk are shown in Table 5.3. In both milking frequencies, the correlations between fat and fatty acids were weak to moderate. The correlations between fat percentage and fatty acids from C4:0 to C12:0 were negative for cows milked TAD, but were not significant for cows milked OAD except for C4:0 and C6:0. Correlation between fat and C16:0, and fat and C18:1 *cis*-9 were weak and positive in both milking frequencies. In cows milked TAD the correlation between fat and solid fat content was significant and positive.

**Table 5.3.** Partial correlation coefficients between fat percentage and fatty acid composition of cows milked once a day (OAD) and twice a day (TAD).

Fatty Acids	Milking Frequency			
	OAD	p-Value	TAD	p-Value
SFA <sup>1</sup>	-0.09	0.0228	-0.04	0.3954
UFA <sup>2</sup>	0.2	<0.0001	0.14	0.0027
PUFA <sup>3</sup>	-0.12	0.0022	-0.11	0.0117
C4:0	-0.15	<0.0001	-0.43	<0.0001
C6:0	-0.12	0.0017	-0.34	<0.0001
C8:0	0.01	0.8817	-0.20	<0.0001
C10:0	-0.02	0.5528	-0.18	<0.0001
C12:0	-0.01	0.7613	-0.11	0.0148
C14:0	-0.04	0.3424	0.04	0.3271
C16:0	0.24	<0.0001	0.32	<0.0001
C18:0	0.06	0.1455	0.23	<0.0001
C18:1 <i>cis</i> -9	0.17	<0.0001	0.11	0.0136
Omega6	-0.21	<0.0001	-0.21	<0.0001
SCFA <sup>4</sup>	-0.13	0.0014	-0.38	<0.0001
MCFA <sup>5</sup>	-0.03	0.4321	-0.03	0.5355
LCFA <sup>6</sup>	0.37	<0.0001	0.45	<0.0001
SFC <sub>10</sub> <sup>7</sup>	0.03	0.5057	0.21	<0.0001

<sup>1</sup> Saturated fatty acids; <sup>2</sup> Unsaturated fatty acid; <sup>3</sup> Polyunsaturated fatty acid; <sup>4</sup> Short-chain fatty acids (sum of C4:0, C6:0 and C8:0); <sup>5</sup> Medium-chain fatty acids (sum of C10:0, C12:0); <sup>6</sup> Long-chain fatty acids (sum of C14:0, C16:0, C18:0, C18:1 *cis*-9 and omega-6); <sup>7</sup> Solid fat content at 10°C.

Least-square means and standard errors for production and composition traits for the three SNP rs109421300 genotypes in both milking frequencies are shown in Table 5.4. Compared with the CC genotype, the TT genotype was significantly ( $p < 0.05$ ) associated with higher milk yield, protein yield, and lactose yield in both milking frequencies whereas the CC genotype was associated with significantly ( $p < 0.05$ ) higher values for fat yield, fat percentage, and protein percentage compared with the TT genotype in both milking frequencies. The CT genotype was intermediate for all these traits. In cows milked OAD and TAD, the TT genotype was associated with significantly ( $p < 0.05$ ) higher proportions of C4:0 and C18:1 *cis*-9 compared with the CC genotype whereas the CC genotype was associated with significantly higher proportions of C16:0 and C18:0 compared with the TT genotype. The CT had intermediate values for the proportions of C16:0 and C18:0.

**Table 5.4.** Least-square means and standard errors for the association between milk production traits, milk composition, and fatty acid composition and SNP rs109421300 genotypes for cows milked once a day (OAD) and twice a day (TAD).

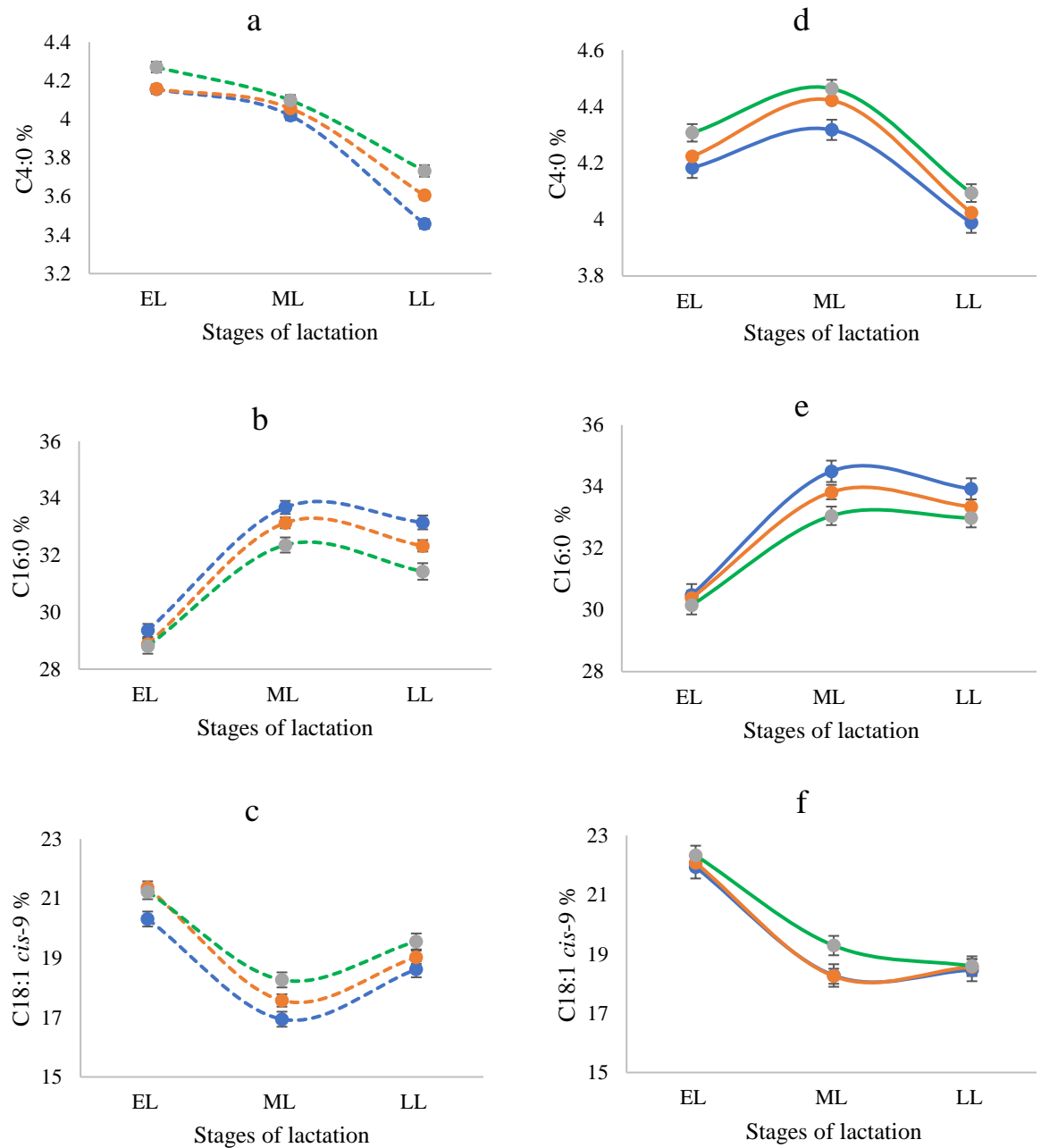
Variables	OAD			TAD			p-Value MF <sup>8</sup> *SNP
	CC	CT	TT	CC	CT	TT	
Milk yield, kg/day	17.07 ± 0.3 <sup>e</sup>	18.78 ± 0.24 <sup>d</sup>	19.4 ± 0.36 <sup>d</sup>	22.84 ± 0.46 <sup>c</sup>	24.27 ± 0.33 <sup>b</sup>	25.75 ± 0.4 <sup>a</sup>	<0.0001
Fat yield, kg/day	0.93 ± 0.02 <sup>b</sup>	0.91 ± 0.01 <sup>b</sup>	0.85 ± 0.02 <sup>c</sup>	1.06 ± 0.03 <sup>a</sup>	1.06 ± 0.02 <sup>a</sup>	1.03 ± 0.02 <sup>a</sup>	0.015
Protein yield, kg/day	0.70 ± 0.01 <sup>d</sup>	0.75 ± 0.01 <sup>c</sup>	0.75 ± 0.01 <sup>c</sup>	0.84 ± 0.02 <sup>b</sup>	0.87 ± 0.01 <sup>ab</sup>	0.89 ± 0.02 <sup>a</sup>	0.0025
Lactose yield, kg/day	0.80 ± 0.02 <sup>e</sup>	0.89 ± 0.01 <sup>e</sup>	0.91 ± 0.02 <sup>d</sup>	1.13 ± 0.02 <sup>c</sup>	1.19 ± 0.02 <sup>b</sup>	1.25 ± 0.02 <sup>a</sup>	<0.0001
Fat %	5.34 ± 0.07 <sup>a</sup>	4.80 ± 0.05 <sup>b</sup>	4.33 ± 0.08 <sup>c</sup>	4.08 ± 0.1 <sup>c</sup>	3.67 ± 0.07 <sup>d</sup>	3.15 ± 0.09 <sup>e</sup>	<0.0001
Protein %	4.27 ± 0.03 <sup>a</sup>	4.15 ± 0.02 <sup>b</sup>	4.05 ± 0.03 <sup>c</sup>	3.84 ± 0.04 <sup>d</sup>	3.74 ± 0.03 <sup>e</sup>	3.60 ± 0.04 <sup>f</sup>	<0.0001
Lactose %	4.80 ± 0.02 <sup>d</sup>	4.85 ± 0.01 <sup>c</sup>	4.79 ± 0.02 <sup>d</sup>	5.07 ± 0.03 <sup>a</sup>	5.04 ± 0.02 <sup>a</sup>	4.97 ± 0.02 <sup>b</sup>	0.0016
Fatty acid, % of the total FA							
SFA <sup>1</sup>	71.4 ± 0.21 <sup>a</sup>	70.51 ± 0.17 <sup>bc</sup>	70.1 ± 0.25 <sup>b</sup>	71.55 ± 0.32 <sup>a</sup>	71.42 ± 0.23 <sup>a</sup>	70.88 ± 0.29 <sup>ac</sup>	0.0016
UFA <sup>2</sup>	29.29 ± 0.18 <sup>b</sup>	29.9 ± 0.14 <sup>a</sup>	30.18 ± 0.21 <sup>a</sup>	28.53 ± 0.27 <sup>c</sup>	28.59 ± 0.19 <sup>c</sup>	28.79 ± 0.24 <sup>bc</sup>	0.0171
PUFA <sup>3</sup>	3.05 ± 0.03 <sup>b</sup>	3.15 ± 0.02 <sup>a</sup>	3.16 ± 0.03 <sup>a</sup>	2.82 ± 0.04 <sup>c</sup>	2.87 ± 0.03 <sup>c</sup>	2.84 ± 0.04 <sup>c</sup>	0.0291
C4:0	3.89 ± 0.02 <sup>e</sup>	3.94 ± 0.02 <sup>d</sup>	4.03 ± 0.02 <sup>c</sup>	4.17 ± 0.03 <sup>b</sup>	4.23 ± 0.02 <sup>b</sup>	4.29 ± 0.03 <sup>a</sup>	<0.0001
C6:0	2.85 ± 0.01 <sup>c</sup>	2.86 ± 0.01 <sup>c</sup>	2.89 ± 0.02 <sup>bc</sup>	2.89 ± 0.02 <sup>bc</sup>	2.93 ± 0.02 <sup>ab</sup>	2.95 ± 0.02 <sup>a</sup>	0.0543
C8:0	1.60 ± 0.01 <sup>a</sup>	1.59 ± 0.01 <sup>ac</sup>	1.59 ± 0.01 <sup>ac</sup>	1.54 ± 0.01 <sup>b</sup>	1.57 ± 0.01 <sup>bc</sup>	1.56 ± 0.01 <sup>bc</sup>	0.4008
C10:0	3.72 ± 0.03 <sup>a</sup>	3.67 ± 0.02 <sup>a</sup>	3.67 ± 0.03 <sup>a</sup>	3.41 ± 0.04 <sup>c</sup>	3.5 ± 0.03 <sup>b</sup>	3.48 ± 0.04 <sup>bc</sup>	0.1831
C12:0	4.12 ± 0.03 <sup>a</sup>	4.05 ± 0.02 <sup>b</sup>	4.03 ± 0.03 <sup>b</sup>	3.77 ± 0.04 <sup>c</sup>	3.81 ± 0.03 <sup>c</sup>	3.80 ± 0.04 <sup>c</sup>	0.133
C14:0	13.31 ± 0.09 <sup>a</sup>	13.06 ± 0.07 <sup>b</sup>	12.77 ± 0.1 <sup>cd</sup>	12.91 ± 0.13 <sup>bc</sup>	12.95 ± 0.09 <sup>bd</sup>	12.63 ± 0.12 <sup>c</sup>	0.0002
C16:0	32.05 ± 0.18 <sup>b</sup>	31.45 ± 0.14 <sup>c</sup>	30.9 ± 0.21 <sup>d</sup>	32.97 ± 0.27 <sup>a</sup>	32.52 ± 0.19 <sup>ab</sup>	32.06 ± 0.24 <sup>b</sup>	<0.0001
C18:0	13.03 ± 0.12 <sup>b</sup>	12.62 ± 0.09 <sup>c</sup>	12.35 ± 0.14 <sup>cd</sup>	13.59 ± 0.18 <sup>a</sup>	13.12 ± 0.13 <sup>bc</sup>	12.82 ± 0.16 <sup>bc</sup>	<0.0001
C18:1 <i>cis</i> -9	18.59 ± 0.18 <sup>d</sup>	19.30 ± 0.15 <sup>bc</sup>	19.65 ± 0.21 <sup>acb</sup>	19.53 ± 0.28 <sup>b</sup>	19.62 ± 0.2 <sup>b</sup>	20.06 ± 0.24 <sup>a</sup>	0.0009
Omega-6	1.66 ± 0.02 <sup>b</sup>	1.73 ± 0.02 <sup>a</sup>	1.77 ± 0.03 <sup>a</sup>	1.58 ± 0.03 <sup>c</sup>	1.61 ± 0.02 <sup>cb</sup>	1.58 ± 0.03 <sup>cb</sup>	0.0168
SCFA <sup>4</sup>	8.33 ± 0.04 <sup>c</sup>	8.39 ± 0.03 <sup>c</sup>	8.51 ± 0.04 <sup>b</sup>	8.60 ± 0.06 <sup>b</sup>	8.72 ± 0.04 <sup>a</sup>	8.81 ± 0.05 <sup>a</sup>	0.0007
MCFA <sup>5</sup>	21.13 ± 0.14 <sup>a</sup>	20.78 ± 0.11 <sup>b</sup>	20.39 ± 0.16 <sup>c</sup>	20.06 ± 0.21 <sup>cd</sup>	20.24 ± 0.15 <sup>cd</sup>	19.88 ± 0.18 <sup>d</sup>	0.0053
LCFA <sup>6</sup>	65.33 ± 0.2 <sup>d</sup>	65.12 ± 0.16 <sup>de</sup>	64.67 ± 0.24 <sup>e</sup>	67.68 ± 0.31 <sup>b</sup>	66.88 ± 0.22 <sup>ac</sup>	66.53 ± 0.27 <sup>c</sup>	0.0048
Milk income, NZD/day	12.58 ± 0.26 <sup>b</sup>	12.50 ± 0.21 <sup>b</sup>	11.74 ± 0.30 <sup>c</sup>	14.59 ± 0.39 <sup>a</sup>	14.67 ± 0.28 <sup>a</sup>	14.61 ± 0.34 <sup>a</sup>	0.2208
SFC <sub>10</sub> <sup>7</sup>	69.83 ± 0.49 <sup>a</sup>	67.49 ± 0.39 <sup>b</sup>	65.03 ± 0.57 <sup>c</sup>	70.45 ± 0.73 <sup>a</sup>	69.21 ± 0.53 <sup>a</sup>	66.89 ± 0.65 <sup>b</sup>	<0.0001

<sup>1</sup> Saturated fatty acids; <sup>2</sup> Unsaturated fatty acids; <sup>3</sup> Polyunsaturated fatty acids; <sup>4</sup> Short-chain fatty acids (sum of C4:0, C6:0 and C8:0); <sup>5</sup> Medium-chain fatty acids (sum of C10:0, C12:0); <sup>6</sup> Long-chain fatty acids (sum of C14:0, C16:0, C18:0, C18:1 *cis*-9 and omega-6); <sup>7</sup> Solid fat content at 10 °C; <sup>8</sup> Milking frequency; Milk income was calculated using the equation NZD 9.78 × kg Fat yield + NZD 8.89 × kg Protein yield – NZD 0.033 × kg milk yield; <sup>a-f</sup> Means with different superscripts between genotypes in cows across milking frequency are significantly different (p < 0.05)

When comparing the two milking frequencies, all three genotypes of cows milked OAD showed significantly lower values for the production traits and significantly higher values for the percentages of fat and protein, compared with the corresponding genotypes of cows milked TAD. In the case of fatty acids C4:0 and C18:1 *cis*-9, cows milked OAD showed significantly lower proportions for all three genotypes than cows milked TAD.

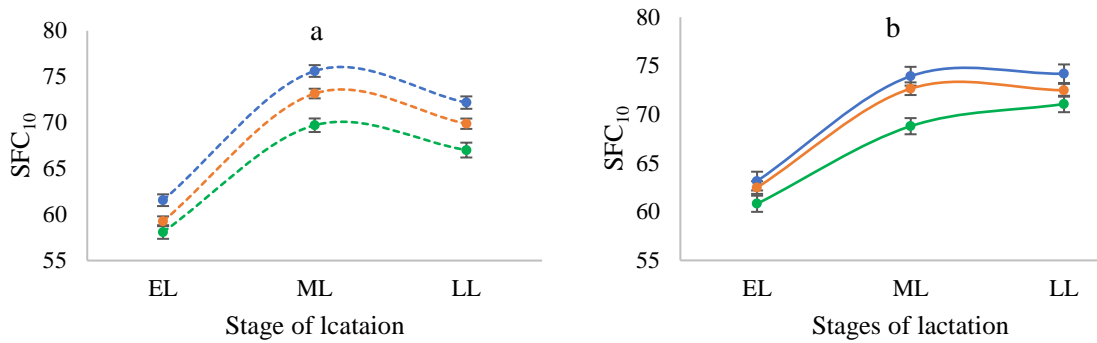
In cows milked OAD, the CC genotype was associated with significantly higher income for milk solids while in cows milked TAD, the genotypes did not significantly differ. The variation in milk income between the two milking frequencies was not significant. Higher SFC<sub>10</sub> was linked to the CC genotype compared with the TT genotype in cows milked OAD and TAD. The genotype CT was intermediate for SFC<sub>10</sub>. When comparing the CC genotype in cows milked TAD, the CC genotype in cows milked OAD was associated with lower SFC<sub>10</sub>.

The three genotypes showed similar patterns throughout the lactation in both milking frequencies with significant differences in the mid- and late lactation stages. In early lactation, the proportions of C4:0 and C18:1 *cis*-9 were higher and the proportion of C16:0 was lower compared with mid- and late lactation in both milking frequencies (Figure 5.2). The SFC<sub>10</sub> was also lower in early lactation in both milking frequencies (Figure 5.3). The genotype TT showed lower SFC<sub>10</sub> throughout the lactation.



**Figure 5.2.** Proportions of (a) C4:0, (b) C16:0, and (c) C18:1 *cis*-9 for genotypes CC (.....), CT (.....), and TT (.....) in cows milked once a day, and proportions of (d) C4:0, (e) C16:0, and (f) C18:1 *cis*-9 for genotypes CC (—), CT (—), and TT (—) in cows milked twice a day during the 2020–2021 production season.

EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.



**Figure 5.3.** Solid fat content at 10°C (SFC<sub>10</sub>) for genotypes CC (....), CT (....), and TT (....) in cows milked once a day (a), and for genotypes CC (—), CT (—), and TT (—) in cows milked twice a day (b) during the 2020–2021 production season. EL = early lactation (<90 days); ML = mid-lactation (90–180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

## 5.5. Discussion

The DGAT1 gene is widely accepted to have the most significant effect on milk production traits [23,26,30]. The SNP rs109421300 selected in this study is located on chromosome 14 in the DGAT1 region at the 1801116 bp position [31]. Lopez-Villalobos et al. [28] reported a significant association of the SNP with the fat percentage in cows milked OAD and TAD. In this study, SNPs in the proximity to the DGAT1 gene were tested for the association for fat percentage and the SNP rs109421300 showed the highest association with fat percentage. In this study, the SNP was significantly associated with the fatty acid composition and SFC<sub>10</sub> of cows milked OAD and TAD.

Few studies have reported a significant effect of the SNP rs109421300 on the milk production traits of cows milked OAD and TAD [28,30]. This SNP has antagonistic pleiotropy between fat yield and milk yield, and fat yield and protein yield [30]. The current study reported the same antagonistic effect in cows milked OAD, with the CC genotype being significantly associated with higher fat yield and lower milk and protein yields. The SNP was positively associated with lower milk and protein yields with no effect on the fat yield in TAD milking. This may be due to the moderate correlation coefficient between fat yield and fat percentage which was 0.43 and is in agreement with Schennink et al. [26] in Dutch Holstein-Friesian cows, and with Lopez-Villalobos et al. [32] in F, F×J, and J cows. In cows milked OAD the correlation coefficient between fat yield and fat percentage was 0.94. The CC genotype was also associated with a significant

positive effect on the fat percentage in both milking frequencies, which is also in agreement with Jiang et al. [30] and Lopez-Villalobos et al. [28]. This could be due to the large negative effect of this SNP on milk yield and the large positive effect on fat yield [30]. This partially agrees with the results of this study, as considerable variation was explained by the SNP for milk yield. Overall, the effect of SNP rs109421300 between fat yield and milk yield was antagonistic, which resulted in a strong and positive effect on fat percentage.

As the SNP rs109421300 was highly associated with fat percentage, the partial correlations between fat and individual fatty acids were analysed. In cows milked TAD, the correlation coefficients between *de novo* synthesised fatty acids (C4:0–C12:0) were negative and weak to moderate. This is partially in agreement with Schennink et al. [26], who reported positive and weak correlations, and Lopez-Villalobos et al. [32], who reported positive and moderated correlations. The positive correlation between fat percentage and C16:0 is in agreement with Schennink et al. [26], Lopez-Villalobos et al. [32], and Soyeurt et al. [33], but the correlations were stronger (0.88, 0.43, and 0.86, respectively) in these studies. The positive and weak correlation between fat percentage and C18:0 is in agreement with Schennink et al. [26] and in contradiction with Lopez-Villalobos et al. [32] and Soyeurt et al. [33], who reported strong positive and moderate negative correlations, respectively. The positive correlation between fat percentage and C18:1 *cis*-9 is in agreement with Soyeurt et al. [33] where the correlation coefficient was higher (0.66) than in the present study. In contrast, Schennink et al. [26] and Lopez-Villalobos et al. [32] reported negative correlations. The positive and significant correlation between fat percentage and SFC<sub>10</sub> in cows milked TAD is in agreement with MacGibbon [19] who reported a positive correlation (0.78). The reason for differences in the correlation coefficients in the above study could be the variation in breed, feed, and management practices.

There are no studies estimating the correlation coefficients between fat percentage and individual fatty acids in milk from cows milked OAD. In the present study, the correlations between fat and fatty acids were weak to moderate. Correlations were in the same directions but in different magnitudes, for fatty acids, C4:0, C6:0, C8:0, C10:0, C12:0, C18:0, SCFA, and SFC<sub>10</sub> in cows milked OAD and TAD. The correlation coefficients were slightly lower for OAD milking compared with TAD milking frequency. In this study, the correlation coefficients between fat percentage and fatty

acids suggest that the SNP could have a less significant effect on individual fatty acids, especially *de novo* synthesis fatty acids compared with the effect on fat percentage. This is also justified by the low and no variance determined for the SNP for these fatty acids except for C4:0. This would be due to the higher affinity of DGAT1 to butyryl-CoA and palmitoyl-CoA [34]. Estimating the genetic variance components for the study traits may explain more about the association of SNP with the traits [35] as estimates of genetic correlations and heritabilities for fatty acids would help find the extent of the same gene affecting different traits.

The CC genotype of the SNP was positively associated with the proportions of C16:0 and C18:0, and negatively associated with the proportion of C4:0, C18:1 *cis*-9, and short-chain fatty acids in both milking frequencies. This suggests that selecting cows for the SNP will lead to a correlated increase in the proportions of C16:0 and C18:0 and a decrease in the proportions of C18:1 *cis*-9 and short-chain fatty acids. This is not optimum for the processability of milk, especially for making butter. Milk with these fatty acids in the above-mentioned proportions would lead to less spreadable butter [19,36]. This is also explained by the higher SFC<sub>10</sub> for the CC genotype, which would tend to produce less spreadable butter. Higher SFC<sub>10</sub> leads to lower spreadability of butter [19,36]. Generally, household consumers prefer softer butter, but some food processing companies, for example, the pastry industry, prefer harder butter to maintain flakiness and crispiness [37]. In New Zealand, cows are selected mainly for higher fat and protein yields. Therefore, selecting cows for the CC genotype would be beneficial for higher income but not for making butter.

In this study, the SNP was not significantly associated with the proportion of *de novo* synthesised fatty acids in both milking frequencies (the effect was very low for proportions of C6:0 and C10:0 for TAD milking). This may be due to the same origin of these fatty acids and similar mechanisms involved in synthesis in the mammary gland. Soyeurt et al. [33] reported high genetic correlations between these fatty acids and explained that the reason could be the similarities in their origin. Knutson et al. [22] reported that some genes in chromosomes 11, 13, 17, and 19 were highly correlated with *de novo* synthesised short- and medium-chain fatty acids (C4:0–C14:0) in cows milked TAD. Overall, the present study reports that although the SNP is highly linked to fat percentage, the association was not stronger for fatty acids. Future research with genome-

wide association studies (GWAS) would help find suitable chromosomes, genes, and SNPs associated with milk composition in cows milked OAD.

The directions of SNP genotypes for most of the traits in this study were similar across milking frequencies. However, the magnitude of the genotypes for some traits behaved differently. The milk yield, fat yield, protein yield, and lactose yield were lower, and fat and protein percentages were higher for all three SNP genotypes in cows milked OAD compared with cows milked TAD. Similarly, the proportions of *de novo* synthesis fatty acids, mainly C6:0–C14:0, were higher, and proportions of long-chain fatty acids were lower in cows milked OAD for all three genotypes compared with cows milked TAD. Similar results were reported by Sanjayaraj et al. [38] in the previous work comparing fatty acid composition between cows milked OAD and TAD.

The variation in the magnitude of the same genotype in different milking frequencies could be due to the interaction between the genotype and the environment. Falconer and Mackay [39] reported that animals would perform differently in diverse environments due to genotype-by-environment interaction. Lopez-Villalobos [40] reported that variation in traits is caused by the effect of genes, environment, and genotype-by-environment interaction. In this study, the expression of SNP genotypes was affected by the milking frequency. The interaction of milking frequency with the mechanism of fatty acid synthesis and the concentration of precursors for fatty acid synthesis [41] could be the factors affecting the expressions of SNP genotypes in different milking frequencies.

Expression of the DGAT1 gene is also affected by the season and stages of lactation [42,43]. In New Zealand, the season and stage of lactation are considered important factors affecting the processability of milk [44]. In this study, all three genotypes showed similar patterns throughout lactation for the fatty acids C4:0, C16:0, C18:1 *cis*-9 and SFC<sub>10</sub> in both milking frequencies. The proportions of these fatty acids and SFC<sub>10</sub> reveal that butter produced in early lactation tends to be easily spreadable, especially with the TT genotype. This is in agreement with Auld et al. [44] who reported that the SFC<sub>10</sub> was lower in early lactation milk compared with milk from the other two stages of lactation. The trends of the curves also show that the spreadability of the butter would tend to decrease in the mid- and late lactation stages, with the TT genotype more likely to produce easily spreadable butter compared with other genotypes. However, these changes are smaller than the overall seasonal changes. In New Zealand, the stage of

lactation is synchronized with the season, pasture quality, and feed availability [18]. Therefore, it is difficult to determine the actual influence of the stage of lactation on the expression of SNP for fatty acids. Auld et al. [44] reported that the effect of the season was greater than the effect of the stage of lactation. The effect of season was not explored in this study. This would need to be confirmed by assessment of the actual product produced.

## 5.6. Conclusions

The CC genotype of SNP rs109421300 was largely and positively associated with fat yield, fat percentage, and protein percentage, and negatively associated with milk and protein yields. The associations of SNP genotypes with fatty acids were similar in both milking frequencies, however, the magnitudes were different due to the differences in the environment. The SNP also had a lower association with the *de novo* synthesised fatty acids in both milking frequencies. The CC genotype was associated with higher proportions of C16:0 and C18:0, lower proportions of C4:0 and C18:1 *cis*-9, and higher SFC<sub>10</sub> in cows milked OAD and TAD, suggesting selecting cows with the CC genotype would lead to the production of butter that would be less spreadable. The CC genotype in cows milked OAD produced lower proportions of C16:0 and C18:0, and lower SFC<sub>10</sub> compared with the same genotype in cows milked TAD. Selecting cows with the CC genotype of SNP rs109421300 could be beneficial for New Zealand dairy farmers with the current payment system while selecting cows with the TT genotype, especially in OAD milking would be beneficial only for butter making. The results of this study pave the way for researchers, farmers, and processing companies to think of a possible way of producing more spreadable butter with OAD milking.

## References

1. Davis, S.R.; Farr, V.C.; Stelwagen, K. Regulation of yield loss and milk composition during once-daily milking: A review. *Livest. Prod. Sci.* **1999**, *59*, 77–94. [https://doi.org/10.1016/S0301-6226\(98\)00204-8](https://doi.org/10.1016/S0301-6226(98)00204-8).
2. Clark, D.A.; Phyn, C.V.C.; Tong, M.J.; Collis, S.J.; Dalley, D.E. A systems comparison of once-versus twice daily milking of pastured dairy cows. *J. Dairy Sci.* **2006**, *89*, 1854–1862. [https://doi.org/10.3168/jds.S0022-0302\(06\)72254-8](https://doi.org/10.3168/jds.S0022-0302(06)72254-8).

3. Edwards, J.P. Comparison of milk production and herd characteristics in New Zealand herds milked once or twice a day. *Anim. Prod. Sci.* **2019**, *59*, 570–580. <https://doi.org/10.1071/AN17484>.
4. Rémond, B.; Pomiès, D.; Dupont, D.; Chilliard, Y. Once-a-day milking of multiparous Holstein cows throughout the entire lactation: Milk yield and composition, and nutritional status. *Anim. Res.* **2004**, *53*, 201–212. <https://doi.org/10.1051/animres:2004014>.
5. Lembeye, F.; López-Villalobos, N.; Burke, J.L.; Davis, S.R. Estimation of breed and heterosis effects for milk traits and somatic cell scores in cows milked once and twice daily in New Zealand. *Proc. N. Z. Soc. Anim. Prod.* **2015**, *75*, 60–63.
6. Sneddon, N.W.; Lopez-Villalobos, N.; Hickson, R.E.; Shalloo, L.; Garrick, D.J. Estimation of crossbreeding effects on yields of dairy products and value of milk processed in different product portfolios. *Proc. N. Z. Soc. Anim. Prod.* **2015**, *75*, 48–53.
7. Dalgleish, D.G. Bovine milk protein properties and the manufacturing quality of milk. *Livest. Prod. Sci.* **1993**, *35*, 75–93. [https://doi.org/10.1016/0301-6226\(93\)90183-I](https://doi.org/10.1016/0301-6226(93)90183-I).
8. Walstra, P.; Wouters, J.T.; Geurts, T.J. *Dairy Science and Technology*; CRC Press, Taylor & Francis: Boca Raton, FL, USA, 2005.
9. MacGibbon, A.K.H. Composition and Structure of Bovine Milk Lipids. In *Advanced Dairy Chemistry*, 4th ed.; McSweeney, P.L.H., Fox, P.F., O'Mahony, J.A., Eds.; Springer: New York, NY, USA, 2020; Volume 2, pp. 1–32.
10. Soyeurt, H.; Dardenne, P.; Gillon, A.; Croquet, C.; Vanderick, S.; Mayeres, P.; Bertozzi, C.; Gengler, N. Variation in fatty acid contents of milk and milk fat within and across breeds. *J. Dairy Sci.* **2006**, *89*, 4858–4865. [https://doi.org/10.3168/jds.S0022-0302\(06\)72534-6](https://doi.org/10.3168/jds.S0022-0302(06)72534-6).
11. Palladino, R.A.; Buckley, F.; Prendiville, R.; Murphy, J.J.; Callan, J.; Kenny, D.A. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. *J. Dairy Sci.* **2010**, *93*, 2176–2184. <https://doi.org/10.3168/jds.2009-2453>.
12. Lopez-Villalobos, N.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Holroyd, S.E.; MacGibbon, A.K.; Snell, R.G. Estimation of genetic and crossbreeding parameters of fatty acid concentrations in milk fat predicted by mid-

- infrared spectroscopy in New Zealand dairy cattle. *J. Dairy Sci.* **2014**, *81*, 340–349. <https://doi.org/10.1017/S0022029914000272>.
13. Back, P.J.; Thomson, N.A. Exploiting cow genotype to increase milk value through production of minor milk components. *Proc. N. Z. Soc. Anim. Prod.* **2005**, *65*, 53–58.
  14. Stoop, W.M.; Bovenhuis, H.; Heck, J.M.L.; van Arendonk, J.A.M. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *J. Dairy Sci.* **2009**, *92*, 1469–1478. <https://doi.org/10.3168/jds.2008-1468>.
  15. Palmquist, D.L.; Beaulieu, A.D.; Barbano, D.M. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* **1993**, *76*, 1753–1771. [https://doi.org/10.3168/jds.S0022-0302\(93\)77508-6](https://doi.org/10.3168/jds.S0022-0302(93)77508-6).
  16. Dewhurst, R.J.; Shingfield, K.J.; Lee, M.R.; Scollan, N.D. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Feed Sci. Technol.* **2006**, *131*, 168–206. <https://doi.org/10.1016/j.anifeedsci.2006.04.016>.
  17. Heck, J.M.; Van Valenberg, H.J.; Dijkstra, J.; Van Hooijdonk, A.C. Seasonal variation in the Dutch bovine raw milk composition. *J. Dairy Sci.* **2009**, *92*, 4745–4755. <https://doi.org/10.3168/jds.2009-2146>.
  18. Schwendel, B.H.; Morel, P.C.; Wester, T.J.; Tavendale, M.H.; Deadman, C.; Fong, B.; Shadbolt, N.M.; Thatcher, A.; Otter, D.E. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. *J. Dairy Sci.* **2015**, *98*, 1411–1425. <https://doi.org/10.3168/jds.2014-8322>.
  19. MacGibbon, A.K. Herd-to-herd variations in the properties of milkfat. *Proc. N. Z. Soc. Anim. Prod.* **1996**, *56*, 224–227.
  20. MacGibbon, A.K.; McLennan, W.D. Hardness of New-Zealand patted butter—seasonal and regional variations. *N. Z. J. Dairy Sci. Technol.* **1987**, *22*, 143–156.
  21. Stoop, W.M.; Schennink, A.; Visker, M.H.P.W.; Mullaart, E.; Van Arendonk, J.A.M.; Bovenhuis, H. Genome-wide scan for bovine milk-fat composition. I. Quantitative trait loci for short-and medium-chain fatty acids. *J. Dairy Sci.* **2009**, *92*, 4664–4675. <https://doi.org/10.3168/jds.2008-1966>.
  22. Knutsen, T.M.; Olsen, H.G.; Tafintseva, V.; Svendsen, M.; Kohler, A.; Kent, M.P.; Lien, S. Unravelling genetic variation underlying *de novo*-synthesis of bovine milk fatty acids. *Sci. Rep.* **2018**, *8*, 2179.

23. Spelman, R.J.; Ford, C.A.; McElhinney, P.; Gregory, G.C.; Snell, R.G. Characterization of the DGAT1 gene in the New Zealand dairy population. *J. Dairy Sci.* **2002**, *85*, 3514–3517. [https://doi.org/10.3168/jds.S0022-0302\(02\)74440-8](https://doi.org/10.3168/jds.S0022-0302(02)74440-8).
24. Bovenhuis, H.; Visker, M.H.; Poulsen, N.A.; Sehested, J.; Van Valenberg, H.J.; Van Arendonk, J.A.; Larsen, L.B.; Buitenhuis, A.J. Effects of the diacylglycerol o-acyltransferase 1 (DGAT1) K232A polymorphism on fatty acid, protein, and mineral composition of dairy cattle milk. *J. Dairy Sci.* **2016**, *99*, 3113–3123. <https://doi.org/10.3168/jds.2015-10462>.
25. Li, Y.; Zhou, H.; Cheng, L.; Edwards, G.R.; Hickford, J.G. Effect of DGAT1 variant (K232A) on milk traits and milk fat composition in outdoor pasture-grazed dairy cattle. *N. Z. J. Agric. Res.* **2021**, *64*, 101–113. <https://doi.org/10.1080/00288233.2019.1589537>.
26. Schennink, A.; Stoop, W.M.; Visker, M.W.; Heck, J.M.; Bovenhuis, H.; Van Der Poel, J.J.; Van Valenberg, H.J.; Van Arendonk, J.A. DGAT1 underlies large genetic variation in milk-fat composition of dairy cows. *Anim. Genet.* **2007**, *38*, 467–473. <https://doi.org/10.1111/j.1365-2052.2007.01635.x>.
27. Tumino, S.; Criscione, A.; Moltisanti, V.; Marletta, D.; Bordonaro, S.; Avondo, M.; Valenti, B. Feeding System Resizes the Effects of DGAT1 Polymorphism on Milk Traits and Fatty Acids Composition in Modicana Cows. *Animals* **2021**, *11*, 1616. <https://doi.org/10.3390/ani11061616>.
28. Lopez-Villalobos, N.; Ariyaratne, H.B.P.C.; Gedye, K.; Correa-Luna, M.; Donaghy, D.J. Association of a SNP in the DGAT1 gene with productive and reproductive performance and profitability in grazing cows milked once and twice a day. *J. Dairy Sci.* **2020**, *103*, 74.
29. Sukhija, P.S.; Palmquist, D.L. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J. Agric. Food Chem.* **1988**, *36*, 1202–1206. <https://doi.org/10.1021/jf00084a019>.
30. Jiang, J.; Ma, L.; Prakapenka, D.; VanRaden, P.M.; Cole, J.B.; Da, Y. A large-scale genome-wide association study in US Holstein cattle. *Front. Genet.* **2019**, *10*, 412. <https://doi.org/10.3389/fgene.2019.00412>.
31. Grisart, B.; Farnir, F.; Karim, L.; Cambisano, N.; Kim, J.J.; Kvasz, A.; Mni, M.; Simon, P.; Frere, J.M.; Coppieters, W.; Georges, M. Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in

- affecting milk yield and composition. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 2398–2403. <https://doi.org/10.1073/pnas.0308518100>
32. Lopez-Villalobos, N.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Sneddon, N.W.; Holroyd, S.E.; MacGibbon, A.K.; Snell, R.G. Genetic correlations of milk fatty acid contents predicted from milk mid-infrared spectra in New Zealand dairy cattle. *J. Dairy Sci.* **2020**, *103*, 7238–7248. <https://doi.org/10.3168/jds.2019-17971>.
33. Soyeurt, H.; Gillon, A.; Vanderick, S.; Mayeres, P.; Bertozzi, C.; Gengler, N. Estimation of heritability and genetic correlations for the major fatty acids in bovine milk. *J. Dairy Sci.* **2007**, *90*, 4435–4442. <https://doi.org/10.3168/jds.2007-0054>.
34. Marshall, M.O.; Knudsen, J. Specificity of diacylglycerol acyltransferase from bovine mammary gland, liver and adipose tissue towards acyl-CoA esters. *Eur. J. Biochem.* **1979**, *94*, 93–98. <https://doi.org/10.1111/j.1432-1033.1979.tb12875.x>.
35. Ariyaratne, H.B.P.C.; Correa-Luna, M.; Blair, H.T.; Garrick, D.J.; Lopez-Villalobos, N. Identification of genomic regions associated with concentrations of milk fat, protein, urea and efficiency of crude protein utilization in grazing dairy cows. *Genes*. **2021**, *12*, 456. <https://doi.org/10.3390/genes12030456>.
36. Mackle, T.R.; Petch, S.F.; Bryant, A.M.; Auldist, M.J.; Henderson, H.V.; MacGibbon, A.K.H. Variation in the characteristics of milkfat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *N. Z. J. Agric. Res.* **1997**, *40*, 349–359. <https://doi.org/10.1080/00288233.1997.9513254>.
37. Kaylegian, K.E. The production of specialty milk fat ingredients. *J. Dairy Sci.* **1999**, *82*, 1433–1439. [https://doi.org/10.3168/jds.S0022-0302\(99\)75370-1](https://doi.org/10.3168/jds.S0022-0302(99)75370-1).
38. Sanjayan, I.; Lopez-Villalobos, N.; Blair, H.T.; Janssen, P.W.M.; Holroyd, S.E.; MacGibbon, A.K.H. Fatty Acid Composition of Dairy Milk: A Case Study Comparing Once- and Twice-a-Day Milking of Pasture-Fed Cows at Different Stages of Lactation. *Dairy*. **2022**, *3*, 174–189. <https://doi.org/10.3390/dairy3010014>.
39. Falconer, D.; Mackay, M. *Introduction to Quantitative Genetics*, 4th ed.; Logman Group: London, UK, 1996; 464p.
40. Lopez-Villalobos, N. Analysing the genetic basis of milk production traits. *CABI Rev.* **2012**, 1–18. <https://doi.org/10.1079/PAVSNNR20127028>.
41. Delamaire, E.; Guinard-Flament, J. Increasing milking intervals decreases the mammary blood flow and mammary uptake of nutrients in dairy cows. *J. Dairy Sci.* **2006**, *89*, 3439–3446. [https://doi.org/10.3168/jds.S0022-0302\(06\)72381-5](https://doi.org/10.3168/jds.S0022-0302(06)72381-5).

42. Bionaz, M.; Periasamy, K.; Rodriguez-Zas, S.L.; Hurley, W.L.; Loor, J.J. A novel dynamic impact approach (DIA) for functional analysis of time-course omics studies: Validation using the bovine mammary transcriptome. *PLoS ONE*. **2012**, *7*, e32455. <https://doi.org/10.1371/journal.pone.0032455>.
43. Kęsek-Woźniak, M.; Wojtas, E.; Zielak-Steciwko, A.E. Impact of SNPs in ACACA, SCD1, and DGAT1 genes on fatty acid profile in bovine milk with regard to lactation phases. *Animals* **2020**, *10*, 997.
44. Auldist, M.J.; Walsh, B.J.; Thomson, N.A. Seasonal and lactational influences on bovine milk composition in New Zealand. *J. Dairy Res.* **1998**, *65*, 401–411. <https://doi.org/10.1017/s0022029998002970>.



## **Foreword to Chapter 6**

Chapter 6 explored the effect of breeds on milk composition and milk coagulation properties in cows milked once a day, twice a day morning, and twice a day afternoon.

In this chapter, 30 cows from the No. 1 Dairy farm (once-a-day farm), and 30 cows from the No. 4 Dairy farm (twice-a-day farm) were selected. Cows selected from both farms included 1<sup>st</sup> and 2<sup>nd</sup> parity Holstein-Friesian, Holstein-Friesian × Jersey and Jersey cows. The breed effect was evaluated with a general linear model that considered breed nested within the milking frequency-milking time.



## Chapter 6

### **A study of milk composition and coagulation properties of Holstein-Friesian, Jersey, and their cross milked once or twice a day**

Published as:

Sanjayanj, I.; Lopez-Villalobos, N.; Blair, H.T.; Janssen, P.W.M.; Holroyd, S.E.; MacGibbon, A.K.H. A study of milk composition and coagulation properties of Holstein-Friesian, Jersey, and their cross milked once or twice a day. *Dairy*. **2023**, *4*, 167–179.  
<https://doi.org/10.3390/dairy4010012>



## 6.1. Abstract

The objective of the study was to explore the effect of breed on the composition and coagulation properties (rennet coagulation time (min), curd firming time (min), and curd firmness (mm)) of milk from cows milked once a day or twice a day in the morning and afternoon, using a Formagraph. Thirty cows (11 Holstein-Friesian, 8 Holstein-Friesian × Jersey, and 11 Jersey) from a once-a-day milking herd and thirty cows (16 Holstein-Friesian, 10 Holstein-Friesian × Jersey, and 4 Jersey) from a twice-a-day milking herd were sampled in late lactation. The milk composition and coagulation properties were analysed for each milk sample. Jersey cows had better milk coagulation properties at each milking frequency-milking time compared with Holstein-Friesian cows. Curd firmness 30 min after the addition of rennet was positively ( $p < 0.05$ ) correlated with the protein percentage. However, the correlations were inconsistent between milking frequencies and milking times, resulting in poor prediction of the changes in cheese-making potential. This study indicated that milk composition and coagulation properties were affected by breed and milking frequency. The effect of the breed could be due to the variation in the composition of the milk, but firm recommendations were hampered by a low number of samples. Further research with larger cow numbers is justified.

Keywords: breed; cow; curd firming; milk composition; milking frequency; milking time

## 6.2. Introduction

New Zealand farmers traditionally milk their cows twice a day (TAD). However, since the late 1990s, the interest in once-a-day (OAD) milking has grown [1] due to several benefits: reduced labour, management, and feed cost, improved labour efficiency, cow health and reproductive performance, and the creation of alternative employment options for farmers [2–4]. However, OAD milking reduces milk production, which leads to economic uncertainty [3]. However, reduced input cost with OAD milking optimised the profit. Once-a-day milking also modifies the milk composition, with higher percentages of fat and protein, and lower percentage of lactose compared with TAD milking [5,6].

Milk coagulation properties (MCPs) are measures of milk processability for cheese making. Typical MCPs are rennet coagulation time (RCT, min), curd firming time or time taken to form 20 mm depth curd ( $K_{20}$ , min), and curd firmness 30 min after rennet addition ( $A_{30}$ , mm) using a Formagraph. The MCP methodology has been reviewed by Troch et al. [7]. In cheese production, milk that aggregates quickly (low RCT) and forms a firm

curd soon after the addition of the clotting enzyme (low  $K_{20}$  and high  $A_{30}$  values) is desirable.

Milk composition affects the yield and quality of dairy products [8]. Milk coagulation properties are crucial factors and basic requirements for cheese making [9]. Fat and protein are the most important milk solids that affect MCPs. Milk protein contributes to the functional properties of coagulation such as gel formation, curd formation, ion binding, and emulsifying ability [8]. Casein is the most important protein component affecting the coagulation properties of milk. Generally, milk with a higher ratio of casein to whey protein is highly suitable for cheese-making [10]. A higher concentration of calcium ions in milk also leads to good quality cheese, aggregating para-casein micelles by reducing the surface charges [10,11].

Selecting breeds that produce milk more suitable for manufacturing dairy products is one way to manipulate milk processing properties [12]. Milk from Jersey cows has higher percentages of fat and protein in both OAD and TAD milking frequencies [12,13]. There are studies in New Zealand reporting the effect of breed on milk composition and MCPs in cows milked TAD [12,14] and international studies reporting the effect of gross composition on the coagulation characteristic of OAD milk [15–17]. However, no studies have been conducted to investigate the effect of breed on the composition and coagulation properties of milk from cows milked OAD and TAD in the morning and afternoon. Studies have also reported differences in the composition of morning and afternoon milking [18,19], which could also affect the MCPs. The objective of this study was to investigate the effect of breed on the composition and coagulation properties of milk from cows milked OAD and TAD in the morning and the evening.

### **6.3. Materials and Methods**

#### ***6.3.1. Animal management and feeding***

This study was conducted at the No.1 Dairy farm and No.4 Dairy farm of Massey University, Palmerston North during the 2020–2021 production season. The No.1 Dairy farm is managed as a low input system with cows milked OAD, and the No.4 Dairy farm is managed as a high input system with cows milked TAD. The details of farm and milking frequencies have previously been given in detail by Sanjayan et al. [20]. The feed composition and chemical composition of the feed given in the production season are shown in Table 6.1.

**Table 6.1.** Dietary and chemical composition of feed offered at the No. 1 and No. 4 Dairy farms during the sampling period (late lactation) in the 2020–2021 production season.

Farm	No. 1 Dairy farm (OAD <sup>12</sup> )	No. 4 Dairy farm (TAD <sup>13</sup> )
Diet composition (kg DM <sup>1</sup> per cow per day)		
Pasture	4.5	10.0
Herb mix crop <sup>2</sup>	3.0	-
Maize silage	-	2.0
DDG <sup>3</sup>	-	-
Tapioca pellets	1.5	-
Concentrate <sup>4</sup>	2.0	1.0
Dry roughage <sup>5</sup>	-	1.0
Baleage <sup>6</sup>	4.0	10.0
Diet chemical composition		
ME <sup>7</sup> MJ/kg DM	9.98	11.28
CP <sup>8</sup> g/100 g DM	21.91	21.27
NDF <sup>9</sup> g/100 g DM	39.88	45.69
ADF <sup>10</sup> g/100 g DM	26.00	25.40
SSS <sup>11</sup> g/100 g DM	8.79	8.59
Lipid g/100 g DM	4.54	4.77

<sup>1</sup> DM = dry matter; <sup>2</sup> Comprises chicory (*Cichorium intybus*), red clover (*Trifolium pratense*), and plantain (*Plantago lanceolata*); <sup>3</sup> dried distillers grains; <sup>4</sup> grain-based concentrate; <sup>5</sup> hay and straw; <sup>6</sup> Lucerne baleage in the No. 1 Dairy farm and grass baleage in the No. 4 Dairy farm; <sup>7</sup> ME = metabolisable energy; <sup>8</sup> CP = crude protein; <sup>9</sup> NDF = neutral detergent fibre; <sup>10</sup> ADF = acid detergent fibre; <sup>11</sup> SSS = soluble sugars and starch; <sup>12</sup> OAD = once-a-day milking; <sup>13</sup> TAD = twice-a-day milking; baleage is partly dried forage, with 45–55% moisture and is baled and wrapped with at least 6 layers of 0.025 mm plastic.

### 6.3.2. Milk sampling

Milk samples were collected from 30 cows (11 Holstein-Friesian (F), 8 Holstein-Friesian × Jersey (F×J), and 11 Jersey (J)) from a OAD milking herd and 30 cows (16 F, 10 F×J, and 4 J) from a TAD milking herd during late lactation ( $229 \pm 13$  days in milk) in the 2020–2021 production season. The sampled cows from No.1 Dairy and No.4 Dairy farms each contained 15 first parity and 15 second parity cows and had no clinical mastitis or metabolic diseases. Morning milk samples were collected from the OAD herd, and morning and afternoon milk samples were collected from the TAD herd separately. Composite milk samples were collected using a Waikato milk flow meter. All samples were refrigerated at 0–4°C without adding any preservatives and analysed within 2 days of sampling.

### 6.3.3. Analysis of milk coagulation properties

The MCPs were measured using a Formagraph [7,21] (Foss Electric, DK-3400, Hillerød, Denmark) of the model type 20: 1 recorder + 1 service module with the capacity of 20 samples/hour. Each sample of 10 mL milk was heated to 35°C and mixed with 0.2 mL rennet solution. Rennet powder (1100 IMCU/g with 96% chymosin and 4% pepsin; prepared according to IDF standard 157:2007 by Renco, New Zealand) was freshly diluted with Milli-Q water to obtain a solution of 0.24% (wt/vol), with a final value of 0.0528 IMCU/mL before addition. Rennet coagulation time (RCT, min), curd firming time or time taken to form 20 mm depth curd ( $K_{20}$ , min), and curd firmness 30 min after rennet addition ( $A_{30}$ , mm) were measured for two replicates for each sample. The Formagraph was used in this study due to the large number of samples, as it allows a minimum of 20 milk samples to coagulate in a maximum of one hour.

The following model was developed to predict the average curd firmness over time for the different milking groups to allow the shape of the curves to be described. This model is a 2<sup>nd</sup> order plus dead time (SOPDT) model [22] to get a better fit early in the response to improve the predictive capability of the model.

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

where  $CF_t$  is the curd firmness at time  $t$  (mm);  $CF_p$  is the asymptotical potential value of curd firmness at an infinite time (mm);  $\tau_1$  and  $\tau_2$  are the time constants;  $RCT_{eq}$  is the rennet coagulation time equivalent to the RCT in traditional Formagraph. This model was only used in Figure 1 to illustrate the average different shapes of the Formagraph plots over time for the different milking groups.

### 6.3.4. Determination of milk composition

Percentages of fat, protein, lactose, and total solids of all the samples were measured using a Milkoscan FT1 instrument (Foss Analytical, Hillerød, Denmark).

### 6.3.5. Statistical analysis

Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Least-square means, and standard errors of the variables were obtained using the following linear model using the GLM procedure.

$$Y_{ijklm} = \mu + MS_i + B_j(MS_i) + L_k + \beta_1 dm + e_{ijklm}$$

$Y_{ijklm}$  is the observation for composition and coagulation traits for milking frequency-milking time  $i$ , breed group  $j$ , lactation number  $k$ , and cow  $m$ .

$\mu$  is the population mean.

$MS_i$  is the fixed effect of milking frequency-milking time  $i$  ( $i = \text{OAD, TAD-morning, and TAD-afternoon}$ ).

$B_j(MS_i)$  is the fixed effect of breed  $j$  nested in milking frequency-milking time  $i$  ( $j = \text{F, F} \times \text{J, and J}$ ).

$L_k$  is the fixed effect of lactation number ( $k = 1^{\text{st}}$  and  $2^{\text{nd}}$  lactation).

$\beta_1$  is the regression coefficient associated with the linear effect of deviation from the median calving date of cow  $m$ .

$e_{ijklm}$  is the residual random error assumed with mean zero and variance  $\sigma_e^2$ .

A limitation of this study was the confounding effect of feed and milking frequency. The feed provided by each farm was not identical during the experimental period. The cows milked TAD were fed slightly higher supplements than cows milked OAD. However, the genetic merit of the cows in the two farms was similar because there were a few sires that had progeny in both farms.

Partial correlations between the dependent variables in each milking frequency-milking time were performed by MANOVA using the GLM procedure of SAS adjusting for the fixed effects of breed and lactation number.

Above GLM model was used with the direct measurements of MCPs from Formagraph such as RCT,  $K_{20}$ , and  $A_{30}$ .

## 6.4. Results

Descriptive statistics of daily milk yield, milk composition, and MCPs of cows sampled in the study are presented in Table 6.2.

**Table 6.2.** Descriptive statistics of milk composition and milk coagulation properties of cows sampled during late lactation in the 2020–2021 production season.

Trait	N	Mean	SD	CV%	Min	Max
Milk yield, kg/day	90	8.68	3.15	37.32	3.40	16.79
Fat %	90	5.32	1.19	22.39	2.62	8.53
Protein %	90	4.38	0.35	7.98	3.62	5.52
Lactose %	90	4.74	0.19	4.00	4.19	5.05
Total solids %	90	15.02	1.40	9.30	11.6	18.53
RCT <sup>1</sup> (min)	82	14.07	4.67	33.17	6.02	25.51
K <sub>20</sub> <sup>2</sup> (min)	76	4.17	2.13	51.05	1.56	10.70
A <sub>30</sub> <sup>3</sup> (mm)	88	40.24	17.13	42.57	0.01	60.34

<sup>1</sup> Rennet coagulation time; <sup>2</sup> Curd firming time; <sup>3</sup> Width of the curd at 30 min after rennet addition.

The minimum value for A<sub>30</sub> was 0.01 mm. A very low value for A<sub>30</sub> was observed for 14% of F cows and 7% of F×J cows. F-values for factors affecting milk yield, milk composition traits, and MCPs are presented in Table 6.3. The milking frequency-milking time explained the largest proportion of variation in most traits, except for lactose percentage, K<sub>20</sub>, and A<sub>30</sub>; for these traits, milking frequency-milking time was non-significant. Breed nested in milking frequency-milking time explained the significant variation in all traits except for lactose percentage. For lactose percentage and A<sub>30</sub>, the lactation number explained the majority of variations.

**Table 6.3.** The F-values for factors affecting milk composition and milk coagulation properties of cows milked once a day and twice a day sampled in the morning and afternoon during the late lactation in the 2020–2021 production season.

Trait	Milking frequency- milking time	Lactation number	Breed within milking frequency-milking Time	dmcd <sup>4</sup>
Milk yield, kg/day	62.5 ***	17.73 ***	4.5 **	1.43
Fat%	26.94 ***	1.23	3.06 *	1.25
Protein %	7.41 **	0.03	3.06 *	0.85
Lactose %	2.08	5.08 *	0.66	0.50
Total solids %	19.05 ***	0.44	3.84 **	1.23
RCT <sup>1</sup> (min)	30.13 ***	2.60	3.59 **	0.28
K <sub>20</sub> <sup>2</sup> (min)	0.09	0.19	3.18 **	0.37
A <sub>30</sub> <sup>3</sup> (mm)	1.60	5.63 *	4.24 **	0.14

<sup>1</sup> Rennet coagulation time; <sup>2</sup> Curd firming time; <sup>3</sup> Width of the curd at 30 min after rennet addition; <sup>4</sup> deviation from median calving date. Statistical significance is given as \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001.

Records with poorly correlated samples (samples showed lower  $A_{30}$ ) were included when calculating the least-square means to avoid bias in the results. The least-square means and standard errors for the milk composition and coagulation properties for F, F×J, and J cows milked OAD or TAD in the morning and afternoon are shown in Table 6.4. Milk from F×J cows milked TAD morning had significantly ( $p < 0.05$ ) higher fat percentage compared with F cows. Jersey cows milked TAD afternoon produced milk with a higher percentage of fat compared with F cows. Similarly, Jersey cows milked OAD and TAD in the morning produced milk with a significantly higher percentage of protein compared with F cows. Milk from J cows coagulated significantly faster (lower RCT and  $K_{20}$  means) and produced a significantly firmer curd (higher  $A_{30}$  mean) compared with F cows in each milking frequency-milking time. The  $K_{20}$  was significantly lower in J cows milked TAD in the morning and afternoon compared with F cows. Friesian × Jersey cows had intermediate values for most MCPs in each milking frequency-milking time, but most differences were not significant.

**Table 6.4.** Least-square means and standard errors of milk composition and coagulation properties of milk from Holstein-Friesian (F), Holstein-Friesian  $\times$  Jersey (F $\times$ J), and Jersey (J) cows milked once a day (OAD) and twice a day (TAD) sampled in the morning and afternoon during the late lactation in the 2020–2021 production season.

Trait	OAD			TAD-morning			TAD-afternoon		
	F	F $\times$ J	J	F	F $\times$ J	J	F	F $\times$ J	J
Milk yield, kg/day	12.07 $\pm$ 0.54 <sup>a</sup>	11.24 $\pm$ 0.71 <sup>ab</sup>	9.53 $\pm$ 0.53 <sup>b</sup>	10.85 $\pm$ 0.44 <sup>a</sup>	8.15 $\pm$ 0.56 <sup>b</sup>	9.17 $\pm$ 0.88 <sup>ab</sup>	5.94 $\pm$ 0.44	4.79 $\pm$ 0.56	5.06 $\pm$ 0.88
Fat %	5.44 $\pm$ 0.27	5.85 $\pm$ 0.35	6.15 $\pm$ 0.26	3.82 $\pm$ 0.22 <sup>b</sup>	4.82 $\pm$ 0.28 <sup>a</sup>	4.44 $\pm$ 0.44 <sup>ab</sup>	5.56 $\pm$ 0.22 <sup>b</sup>	6.13 $\pm$ 0.28 <sup>ab</sup>	6.82 $\pm$ 0.44 <sup>a</sup>
Protein %	4.45 $\pm$ 0.09 <sup>b</sup>	4.57 $\pm$ 0.12 <sup>ab</sup>	4.81 $\pm$ 0.09 <sup>a</sup>	4.15 $\pm$ 0.08 <sup>b</sup>	4.29 $\pm$ 0.09 <sup>ab</sup>	4.61 $\pm$ 0.15 <sup>a</sup>	4.20 $\pm$ 0.08	4.35 $\pm$ 0.09	4.41 $\pm$ 0.15
Lactose %	4.66 $\pm$ 0.06	4.73 $\pm$ 0.07	4.67 $\pm$ 0.06	4.82 $\pm$ 0.05	4.72 $\pm$ 0.06	4.84 $\pm$ 0.09	4.76 $\pm$ 0.05	4.70 $\pm$ 0.06	4.78 $\pm$ 0.09
Total solids %	15.13 $\pm$ 0.33 <sup>b</sup>	15.72 $\pm$ 0.43 <sup>ab</sup>	16.28 $\pm$ 0.32 <sup>a</sup>	13.29 $\pm$ 0.27 <sup>b</sup>	14.47 $\pm$ 0.34 <sup>a</sup>	14.52 $\pm$ 0.53 <sup>a</sup>	15.02 $\pm$ 0.27 <sup>b</sup>	15.81 $\pm$ 0.34 <sup>ab</sup>	16.63 $\pm$ 0.53 <sup>a</sup>
RCT <sup>1</sup> (min)	18.33 $\pm$ 1.04 <sup>a</sup>	17.19 $\pm$ 1.37 <sup>ab</sup>	14.1 $\pm$ 1.02 <sup>b</sup>	11.15 $\pm$ 0.92 <sup>a</sup>	9.49 $\pm$ 1.12 <sup>ab</sup>	6.84 $\pm$ 1.69 <sup>b</sup>	17.18 $\pm$ 0.95 <sup>a</sup>	15.49 $\pm$ 1.20 <sup>ab</sup>	11.74 $\pm$ 1.69 <sup>b</sup>
K <sub>20</sub> <sup>2</sup> (min)	4.16 $\pm$ 0.69	4.79 $\pm$ 0.81	2.81 $\pm$ 0.6	5.39 $\pm$ 0.56 <sup>a</sup>	3.9 $\pm$ 0.66 <sup>ab</sup>	2.01 $\pm$ 1.00 <sup>b</sup>	5.39 $\pm$ 0.61 <sup>a</sup>	3.88 $\pm$ 0.76 <sup>ab</sup>	2.86 $\pm$ 1.00 <sup>b</sup>
A <sub>30</sub> <sup>3</sup> (mm)	34.74 $\pm$ 4.68 <sup>b</sup>	38.38 $\pm$ 6.14 <sup>ab</sup>	53.57 $\pm$ 4.57 <sup>a</sup>	38.52 $\pm$ 3.96 <sup>b</sup>	44.5 $\pm$ 4.81 <sup>ab</sup>	60.08 $\pm$ 7.58 <sup>a</sup>	27.8 $\pm$ 3.83 <sup>b</sup>	36.33 $\pm$ 5.06 <sup>b</sup>	54.91 $\pm$ 7.58 <sup>a</sup>

<sup>1</sup> Rennet coagulation time; <sup>2</sup> Curd firming time; <sup>3</sup> Width of the curd at 30 min after rennet addition; <sup>a,b</sup> Means with different superscripts are significantly different ( $p < 0.05$ ) across breeds within milking frequency-milking time.

In cows milked OAD, all three coagulation properties showed no significant correlation with fat percentage (Table 6.5). However, the correlation between  $K_{20}$  and protein percentage was negative ( $p < 0.05$ ). The correlation between  $A_{30}$  and lactose percentage was positive ( $p < 0.05$ ). The results also show a strong correlation coefficient among MCPs, with RCT being positively and significantly correlated with  $K_{20}$  and negatively and significantly correlated with  $A_{30}$ .

**Table 6.5.** Partial correlation coefficients between coagulation properties and milk components of cows milked once a day during the late lactation in the 2020–2021 production season.

	Milk yield	Fat	Protein	Lactose	Total solids	RCT	$K_{20}$	$A_{30}$
Milk yield, kg/day		-0.38	0.02	0.48 *	-0.30	-0.31	0.10	0.24
Fat %			0.51 *	-0.04	0.97 ***	0.18	-0.34	0.05
Protein %				0.14	0.68 **	0.05	-0.49 *	0.20
Lactose %					0.09	-0.33	-0.11	0.41 *
Total solids %						0.14	-0.42 *	0.13
RCT <sup>1</sup> (min)							0.58 **	-0.89 ***
$K_{20}$ <sup>2</sup> (min)								-0.73 ***
$A_{30}$ <sup>3</sup> (mm)								

<sup>1</sup> Rennet coagulation time; <sup>2</sup> Curd firming time; <sup>3</sup> Width of the curd at 30 min after rennet addition; Statistical significance is given as \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

Table 6.6 shows the partial correlation coefficients between coagulation properties and milk components of cows milked twice a day morning and afternoon. In cows milked TAD in the morning, RCT was negatively ( $p < 0.05$ ) correlated with fat percentage. The time taken to form a 20 mm curd width after rennet addition ( $K_{20}$ ) was significantly and negatively correlated with the percentages of fat and protein, whereas  $A_{30}$  was significantly and positively correlated with fat and protein percentages. In cows milked TAD in the morning and afternoon, the correlation between RCT and  $K_{20}$  was positive ( $p < 0.05$ ) and the correlation between RCT and  $A_{30}$  was strongly negative ( $p < 0.05$ ).

**Table 6.6.** Partial correlation coefficients between coagulation properties and milk components of cows milked twice a day morning (above the diagonal) and afternoon (below the diagonal) during the late lactation in the 2020–2021 production season.

	Milk yield	Fat	Protein	Lactose	Total solids	RCT	K <sub>20</sub>	A <sub>30</sub>
Milk yield, kg/day		-0.22	-0.27	0.50 *	-0.22	0.11	0.06	-0.11
Fat %	-0.29		0.34	-0.03	0.93 ***	-0.67 **	-0.62 **	0.60 **
Protein %	0.04	0.64 **		-0.22	0.64 **	-0.27	-0.50 *	0.53 *
Lactose %	0.39	-0.45	-0.44		-0.03	0.18	0.18	-0.23
Total solids %	-0.20	0.96 ***	0.80 ***	-0.41		-0.65 **	-0.68 **	0.67 **
RCT <sup>1</sup> (min)	0.20	-0.39	0.06	0.02	-0.30		0.89 ***	-0.91 ***
K <sub>20</sub> <sup>2</sup> (min)	-0.02	-0.52 *	-0.32	0.26	-0.49 *	0.71 *		-0.98 ***
A <sub>30</sub> <sup>3</sup> (mm)	-0.18	0.54 *	0.24	-0.25	0.50 *	-0.90 ***	-0.88 ***	

<sup>1</sup> Rennet coagulation time; <sup>2</sup> Curd firming time; <sup>3</sup> Width of the curd at 30 min after rennet addition; Statistical significance is given as \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001.

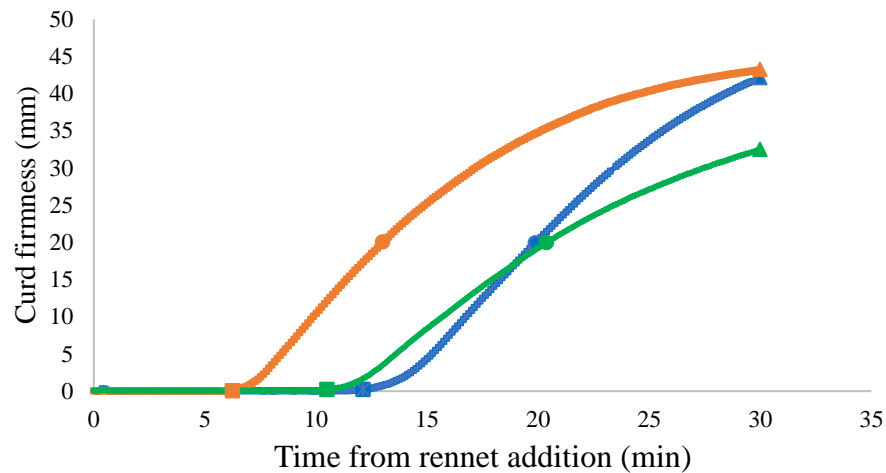
The partial correlation coefficients between coagulation properties and milk components of cows milked in the study are presented in Table 6.7. Curd firming time (K<sub>20</sub>) was significantly and negatively correlated with the percentages of fat and protein whereas A<sub>30</sub> was significantly and positively correlated with fat and protein percentages.

**Table 6.7.** Partial correlation coefficients between coagulation properties and milk components of cows (regardless of milking frequency and milking time) during the late lactation in the 2020–2021 production season.

	Milk Yield	Fat	Protein	Lactose	Total Solids	RCT	K <sub>20</sub>	A <sub>30</sub>
Milk yield, kg/day		-0.27 *	-0.05	0.42 **	-0.21	-0.06	0.06	0.03
Fat %			0.49 ***	-0.10	0.95 ***	-0.21	-0.43 **	0.34 **
Protein %				-0.14	0.70 ***	-0.05	-0.41 **	0.33 **
Lactose %					-0.04	-0.05	0.11	-0.01
Total solids %						-0.21	-0.47 ***	0.40 **
RCT <sup>1</sup> (min)							0.73 ***	-0.89 ***
K <sub>20</sub> <sup>2</sup> (min)								-0.86 ***
A <sub>30</sub> <sup>3</sup> (mm)								

<sup>1</sup> Rennet coagulation time; <sup>2</sup> Curd firming time; <sup>3</sup> Width of the curd at 30 min after rennet addition; Statistical significance is given as \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001.

Figure 6.1 shows the differences between the curd firmness over time from the rennet addition of milk from cows milked OAD and cows milked TAD in the morning and afternoon. The curves were obtained by averaging the curd firmness as measured by the Formagraph. Twice-a-day morning milk coagulated faster than TAD afternoon milk and OAD milk.



**Figure 6.1.** The pattern of curd firming after rennet addition of milk from cows milked once a day (—), twice a day morning (—) and twice a day afternoon (—) in the late lactation in the 2020–2021 production season.

However, as per the model developed, the asymptotical potential value of curd firmness at an infinite time was higher for OAD milk (53.05 mm) compared with TAD-morning milk (47.69 mm). The asymptotical potential value of curd firmness for TAD-afternoon milk was 43.50 mm.

## 6.5. Discussion

The present study evaluated the effect of breed (F, F×J, and J cows) and milking frequency-milking time (OAD and TAD in the morning and afternoon in late lactation) on milk composition and coagulation parameters. It has been previously identified that breed significantly affects MCPs [23,24]. However, little information is available regarding the effects of the breed with the interaction of milking frequency on MCPs.

Jersey cows were superior in the percentages of fat and protein compared with F cows in each milk milking frequency-milking time. These results are in agreement with Auld et al. [12] and Lopez-Villalobos et al. [25] in cows milked TAD, and Tong et al. [3] and

Clark et al. [6] in cows milked OAD. Higher percentages of fat and protein could be a reason for faster  $K_{20}$  and greater curd firmness ( $A_{30}$ ) in J cows compared with F cows within the milking frequency-milking time. Similar results were reported by Auld et al. [12], Poulson et al. [26], and Stocco et al. [27]. Jersey cows were superior for MCPs compared with F cows in each milking frequency-milking time, with F×J cows being intermediate in most cases. A higher percentage of fat in F×J cows milked TAD morning could be due to the heterosis effect in which the progeny performs better than the average performance of the parents.

The fat percentage of milk is mainly associated with curd firmness as in the present study. Huppertz and Kelly [28] reported that milk flocculation occurs due to the collision and aggregation of fat globules, and Stocco et al. [29] reported that this could affect the RCT. The role of fat in milk is considered as an inert filler between the gel network formed by the casein micelles and contributes to the curd structure [29–31]. More accurately, the ratio of casein: fat is more important for the coagulum strength than the percentage of fat on its own [10]. A higher percentage of protein means a higher percentage of casein as 80% of the protein in milk is casein. Milk with a higher concentration of casein leads to the formation of curd with greater firmness [14,32]. Of the different casein fractions,  $\kappa$ -casein is the fraction that initiates the process of cheese-making by interacting with chymosin. When the concentration of  $\kappa$ -casein is higher, the  $\kappa$ -casein hydrolysed by the rennet enzyme would be higher, which leads to the aggregation of more hydrolysed casein and results in a firmer curd. The results of this study suggest that the differences in the MCPs of breeds could be mainly due to the variation in the percentages of fat and protein between the breeds. Auld et al. [12] reported that greater curd firmness in milk from J cows was obvious compared with F cows, in milk from individual cows, in bulk milk, and in milk standardised for protein: fat. However, this difference disappeared when bulk milk was standardised for constant solid concentration. Similarly, Stocco et al. [27] reported, that the differences between breeds for MCPs were mainly due to the variation in the genetic factors controlling the production of milk fat, protein, and lactose. This study suggests the same.

In this study correlations between milk composition and MCPs and correlations among MCPs were obtained. When considering MCPs, strong and negative correlations between RCT and  $A_{30}$  were reported, which was in agreement with Okigbo et al. [33]. Lower RCT means the presence of a higher percentage of protein, especially casein, to form the three-

dimensional network and the gel faster which leads to firmer curd. Rennet coagulation time also positively correlated with  $K_{20}$ , meaning when RCT is lower the formation of a three-dimensional network and gel is faster, therefore the time taken to form 20 mm width curd ( $A_{20}$ ) will be lower. When the percentages of fat and protein were higher,  $K_{20}$  was faster due to the availability of more hydrolysed casein aggregation and trapped fat inside the gel network. However, all these processes depend on the substrate-to-enzyme ratio.

The correlation coefficients between MCPs and the percentages of fat and protein were weak to moderate in each milking frequency-milking time. Rennet coagulation time was not related to percentages of fat or protein except in cows milked TAD in the morning, with higher fat percentage associated with lower RCT. It seems the relationship between  $K_{20}$ , and milk composition depends on the milking frequency-milking time because  $K_{20}$  was correlated with the percentage of protein in OAD milk, correlated with the percentage of fat in TAD afternoon milk, and correlated with both the percentages of fat and protein in TAD morning milk and in all the milk samples regardless of milking frequency and milking time. Similarly, the higher the percentage of either fat or protein or both, the greater the  $A_{30}$ , except for cows milked OAD. This suggests the composition has little effect on MCPs, but they were influenced by milking frequency-milking time.

Similar results were reported by Auld et al. [12] in cows milked TAD, in which RCT was not correlated with any of the milk components, and the correlation coefficients for  $K_{20}$  and  $A_{30}$  were weak. Stocco et al. [29] reported that an increase in the fat percentage in goat milk shortened the RCT and curd firming time and affected the  $A_{45}$  and  $A_{60}$  in a cubic trend. They also reported that a higher percentage of protein increased the RCT and that a higher percentage of casein raised the curd firmness. However, the authors noted that the correlations explained only a small proportion of the variation in the MCPs. De Marchi et al. [23] reported that the percentages of fat and protein did not control significant variation in MCPs as the MCPs were better in the Rendena breed than Brown Swiss, although the Brown Swiss breed had better milk quality traits, particularly higher fat and protein percentages. These results suggest that the composition of milk is not a useful indicator of MCPs of milk from cows milked OAD and TAD in the morning and afternoon. This is also supported by the weak to moderate correlation coefficients obtained in this study. It seems the correlation coefficients depended on milking frequency-milking time as the values of the correlation coefficients between milk composition and MCPs were not constant across milking frequency-milking time

combinations. Keffored et al. [34] and Auldism et al. [12] reported that MCPs are controlled by a more complex combination of parameters than the composition, breed, and protein polymorphism.

One of the important factors affecting MCPs is the genetic variant of  $\kappa$ -casein which can be A or B. The B variant is associated with a higher percentage of protein and a higher proportion of  $\kappa$ -casein in the total casein and leads to improved MCPs [35–37]. In New Zealand and across the world, the A variant of  $\kappa$ -casein is predominant in F cows and the B variant is predominant in J cows [38]. The superiority of J cows for the MCPs could be due to the presence of the B variant for  $\kappa$ -casein as the B allele is associated with greater MCPs [10]. Gai et al. [39] reported that the C variant of  $\alpha$ -casein, B and A1 variants of  $\beta$ -casein, and B variants of  $\beta$ -lactoglobulin are superior for MCPs. Similarly, Walsh et al. [40] and Mayer et al. [41] reported that both the  $\kappa$ -casein BB genotype and  $\beta$ -lactoglobulin BB genotype have greater MCPs. In addition, the calcium concentration of milk is also crucial for better MCPs. Adding  $\text{Ca}^{2+}$  speeds up the RCT by increasing the aggregation of renneted micelles [42]. Davis et al. [43] reported that J cow milk contains a higher Ca: protein ratio. However, Auldism et al. [12] did not observe this. In the present study, the proportions of each casein fraction and calcium were not studied. Milk coagulation is a process that seems dependent on milk composition, mineral composition of milk, genetic variants, and possibly a combination of all factors.

Based on the Formagraph results, the TAD morning milk coagulated faster and formed firmer curd at around 30 min compared with OAD milk and TAD afternoon milk, but the asymptotical potential value of curd firmness was lower compared with OAD milk. Faster rennet coagulation could be explained by the substrate: enzyme ratio. The ratio of  $\kappa$ -casein: rennet would be lower in TAD morning milk and the proportion of cleaved substrate rose faster to form firm curd. On the other hand, OAD milk has a higher concentration of casein [16,17] and therefore, a higher  $\kappa$ -casein: rennet ratio, which led to the proportion of cleaved substrate initially rising more slowly (i.e., longer RCT) but the formation of a more extensive network in a given sufficient time and hence a higher asymptotical potential value than the TAD morning milk. Stocco et al. [27] reported that in goat milk, where the concentration of rennet was not adjusted to the concentration of protein, the RCT was longer due to the lower rennet-to-casein ratio. The reason for this is the need for more time to form sufficient hydrolysis of  $\kappa$ -casein to induce the aggregation of para-casein micelles [44]. Milk from the OAD herd had higher fat and

protein percentages compared with TAD morning and afternoon milk. As adding a constant amount of rennet gives a higher substrate: enzyme ratio for higher protein milk, some of the observed results may indicate that the MCPs, as measured by the Formagraph, are being affected by the substrate: enzyme ratio. Therefore, further studies should be conducted while keeping the substrate: enzyme ratio constant.

Low values for  $A_{30}$  could be due to the poor coagulation ability of milk. De Marchi et al. [23] reported F cows showed the highest percentage of poorly coagulated samples compared with Simmental and Rendena cows in Italy. Tyriseva et al. [45] found that noncoagulating milk from F cows was genetically related to each other and suggested that these poor MCPs could be linked to genetic factors. Chiofaloa et al. [46] proposed that the reason for lower MCPs in F cows could be a higher concentration of plasmin content, which causes proteolysis of casein and partially reduce the casein: true protein ratio. The limitation of using the current Formagraph method is that the time of the coagulation process is set to 30 min. This causes failure in measuring the RCT and  $K_{20}$  values [27]. The use of traditional Formagraph test time could introduce a bias in comparing milk [27] from different breeds and different milking frequency-milking times. The Formagraph is a useful tool to measure the MCPs for 30 min, and models were developed to predict the curd firmness beyond 30 min using the information from the Formagraph [47].

The results of the present study were obtained in the late lactation of the production season. Lucey [48] reported that milk from late lactation is less suitable for producing high-quality dairy products. This could be due to higher plasmin and plasmin activity which cause the deterioration of MCPs by the proteolysis of casein in milk [49]. Lucey [48] reported that OAD milking during late lactation would produce milk with higher plasminogen and plasmin activity. This suggests that milk from cows milked OAD in late lactation is less suitable for cheese making. However, Bastian et al. [50] reported that plasmin activity did not influence the clotting time. In contrast, Tyriseva et al. [45] and Vacca et al. [51] reported better MCPs in late lactation milk. It is also important to note that the stage of lactation has a strong connection with the quality of the diet. Therefore, the quality and processability of late lactation milk are also decided by the diet given to cows. The study could be repeated using cows in early and mid-lactation with controlled feed to find the suitability of milk for processing from different breeds and milking frequency.

## 6.6. Conclusions

Jersey cows were superior for the fat and protein percentages and MCPs compared with F cows milked in OAD milk, TAD morning milk and TAD afternoon milk. While milk composition was correlated with MCPs, they were only low to moderate and unlikely to help predict MCPs. Better relationships between the percentage of fat and MCPs, and the percentage of protein and MCPs were observed in TAD morning milk. There were differences in MCPs between breeds, but this was likely due to the composition of milk. Milk coagulation properties seem to be controlled by a combination of several factors. Selecting J cows would be beneficial for processing companies as they show greater MCPs based on the concentration of milk solids. However, further research with more control on feeding and milk composition would lead to a better understanding of the true effect of breeds and milking frequency on MCPs.

## References

1. Davis, S.R. Lactational traits of importance in dairy cows and applications for emerging biotechnologies. *N Z Vet. J.* **2005**, *53*, 400–405. <https://doi.org/10.1080/00480169.2005.36584>.
2. Davis, S.R.; Farr, V.C.; Stelwagen, K. Regulation of yield loss and milk composition during once-daily milking: A review. *Livest. Prod. Sci.* **1999**, *59*, 77–94. [https://doi.org/10.1016/S0301-6226\(98\)00204-8](https://doi.org/10.1016/S0301-6226(98)00204-8).
3. Tong, M.J.; Clark, D.A.; Cooper, C.V. Once-a-day milking: Possible and profitable? *Proc. N. Z. Grassl. Assoc.* **2002**, *64*, 33–37. <https://doi.org/10.33584/jnzg.2002.64.2470>.
4. Stelwagen, K.; Phyn, C.V.V.; Davis, S.R.; Guinard-Flament, J.; Pomiès, D.; Roche, J.R.; Kay, J.K. Invited review: Reduced milking frequency: Milk production and management implications. *J. Dairy Sci.* **2013**, *96*, 3401–3413. <https://doi.org/10.3168/jds.2012-6074>.
5. Holmes, C.W.; Wilson, G.F.; Mackenzie, D.D.; Purchas, J. The effects of milking once-daily throughout lactation on the performance of dairy cows grazing on pasture. *Proc. N. Z. Soc. Anim. Prod.* **2002**, *52*, 13–16.
6. Clark, D.A.; Phyn, C.V.C.; Tong, M.J.; Collis, S.J.; Dalley, D.E. A systems comparison of once-versus twice daily milking of pastured dairy cows. *J. Dairy Sci.* **2006**, *89*, 1854–1862. [https://doi.org/10.3168/jds.S0022-0302\(06\)72254-8](https://doi.org/10.3168/jds.S0022-0302(06)72254-8).

7. Troch, T.; Lefebure, E.; Baeten, V.; Colinet, F.; Gengler, N.; Sindic, M. Cow milk coagulation: Process description, variation factors and evaluation methodologies. A review. *Biotechnol. Agron. Société Et Environ.* **2017**, *21*, 276–287. <https://doi.org/10.25518/1780-4507.13692>.
8. Dalgleish, D.G. Bovine milk protein properties and the manufacturing quality of milk. *Livest. Prod. Sci.* **1993**, *35*, 75–93. [https://doi.org/10.1016/0301-6226\(93\)90183-I](https://doi.org/10.1016/0301-6226(93)90183-I).
9. Summer, A.; Malacarne, M.; Martuzzi, F.; Mariani, P. Structural and functional properties of Modenese cow milk in Parmigiano-Reggiano cheese production. *Ann. Fac. Med. Vet. Univ. Parma.* **2002**, *22*, 163–174.
10. Amenu, B.; Deeth, H.C. The impact of milk composition on cheddar cheese manufacture. *Aust. J. Dairy Technol.* **2007**, *62*, 171–184.
11. Stocco, G.; Summer, A.; Cipolat-Gotet, C.; Malacarne, M.; Cecchinato, A.; Amalfitano, N.; Bittante, G. The mineral profile affects the coagulation pattern and cheese-making efficiency of bovine milk. *J. Dairy Sci.* **2021**, *104*, 8439–8453. <https://doi.org/10.3168/jds.2021-20233>.
12. Auldist, M.J.; Johnston, K.A.; White, N.J.; Fitzsimons, W.P.; Boland, M.J. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *J. Dairy Sci.* **2004**, *71*, 51–57. <https://doi.org/10.1017/s0022029903006575>.
13. Lembeye, F.; López-Villalobos, N.; Burke, J.L.; Davis, S.R. Estimation of breed and heterosis effects for milk traits and somatic cell scores in cows milked once and twice daily in New Zealand. *Proc. N. Z. Soc. Anim. Prod.* **2015**, *75*, 60–63.
14. Auldist, M.J.; Mullins, C.; O'Brien, B.; O'Kennedy, B.T.; Guinee, T. Effect of cow breed on milk coagulation properties. *Milchwissenschaft* **2002**, *57*, 140–143.
15. O'Brien, B.; Ryan, G.; Meaney, W.J.; McDonagh, D.; Kelly, A. Effect of frequency of milking on yield, composition, and processing quality of milk. *J. Dairy Res.* **2002**, *69*, 367–374. <https://doi.org/10.1017/S0022029902005605>.
16. Pomies, D.; Martin, B.; Chilliard, Y.; Pradel, P.; Rémond, B. Once-a-day milking of Holstein and Montbéliarde cows for 7 weeks in mid-lactation. *Animal* **2007**, *1*, 1497–1505. <https://doi.org/10.1017/S1751731107000778>.
17. Martin, B.; Pomies, D.; Pradel, P.; Verdier-Metz, I.; Rémond, B. Yield and sensory properties of cheese made with milk from Holstein or Montbéliarde cows milked

- twice or once daily. *J. Dairy Sci.* **2009**, *92*, 4730–4737. <https://doi.org/10.3168/jds.2008-1914>.
18. Gilbert, G.R.; Hargrove, G.L.; Kroger, M. Diurnal variations in milk yield, fat yield, milk fat percentage, and milk protein percentage of Holstein-Friesian cows. *J. Dairy Sci.* **1973**, *56*, 409–410. [https://doi.org/10.3168/jds.S0022-0302\(73\)85187-2](https://doi.org/10.3168/jds.S0022-0302(73)85187-2).
  19. Bondan, C.; Folchini, J.A.; Noro, M.; Machado, K.M.; Muhls, E.; González, F.H. Variation of cow's milk composition across different daily milking sessions and feasibility of using a composite sampling. *Cienc. Rural.* **2019**, *49*, 1–6. <https://doi.org/10.1590/0103-8478cr20181004>.
  20. Sanjayanj, I.; Lopez-Villalobos, N.; Blair, H.T.; Janssen, P.W.; Holroyd, S.E.; MacGibbon, A.K. Fatty Acid Composition of Dairy Milk: A Case Study Comparing Once-and Twice-a-Day Milking of Pasture-Fed Cows at Different Stages of Lactation. *Dairy* **2022**, *3*, 174–189. <https://doi.org/10.3390/dairy3010014>.
  21. McMahan, D.J.; Brown, R.J. Evaluation of Formagraph for comparing rennet solutions. *J. Dairy Sci.* **1982**, *65*, 1639–1642. [https://doi.org/10.3168/jds.S0022-0302\(82\)82390-4](https://doi.org/10.3168/jds.S0022-0302(82)82390-4).
  22. Seborg, D.E.; Edgar, T.F.; Mellichamp, D.A.; Doyle, F.J., III. *Process Dynamics and Control*, 4th ed.; John Wiley and Sons Inc: Hoboken, NJ, USA, 2016.
  23. De Marchi, M.; Dal Zotto, R.; Cassandro, M.; Bittante, G. Milk coagulation ability of five dairy cattle breeds. *J. Dairy Sci.* **2007**, *90*, 3986–3992. <https://doi.org/10.3168/jds.2006-627>.
  24. Bittante, G.; Penasa, M.; Cecchinato, A. Invited review: Genetics and modeling of milk coagulation properties. *J. Dairy Sci.* **2012**, *95*, 6843–6870. <https://doi.org/10.3168/jds.2012-5507>.
  25. Lopez-Villalobos, N.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Holroyd, S.E.; MacGibbon, A.K.; Snell, R.G. Estimation of genetic and crossbreeding parameters of fatty acid concentrations in milk fat predicted by mid-infrared spectroscopy in New Zealand dairy cattle. *J. Dairy Sci.* **2014**, *81*, 340–349. <https://doi.org/10.1017/S0022029914000272>.
  26. Poulsen, N.A.; Bertelsen, H.P.; Jensen, H.B.; Gustavsson, F.; Glantz, M.; Månsson, H.L.; Andrén, A.; Paulsson, M.; Bendixen, C.; Buitenhuis, A.J.; et al. The occurrence of noncoagulating milk and the association of bovine milk coagulation properties with genetic variants of the caseins in 3 Scandinavian dairy breeds. *J. Dairy Sci.* **2013**, *96*, 4830–4842. <https://doi.org/10.3168/jds.2012-6422>.

27. Stocco, G.; Cipolat-Gotet, C.; Bobbo, T.; Cecchinato, A.; Bittante, G. Breed of cow and herd productivity affect milk composition and modeling of coagulation, curd firming, and syneresis. *J. Dairy Sci.* **2017**, *100*, 129–145. <https://doi.org/10.3168/jds.2016-11662>.
28. Huppertz, T.; Kelly, A.L. Physical chemistry of milk fat globules. In *Advanced Dairy Chemistry, Lipids*, 3rd ed.; Fox, P.F., McSweeney, P.L.H., Eds.; Springer: New York, NY, USA, 2006; Volume 2, pp. 173–212.
29. Stocco, G.; Pazzola, M.; Dettori, M.L.; Paschino, P.; Bittante, G.; Vacca, G.M. Effect of composition on coagulation, curd firming, and syneresis of goat milk. *J. Dairy Sci.* **2018**, *101*, 9693–9702. <https://doi.org/10.3168/jds.2018-15027>.
30. Green, M.L.; Grandison, A.S. Secondary (non-enzymatic) phase of rennet coagulation and postcoagulation phenomena. In *Cheese: Chemistry, Physics and Microbiology*, 2nd ed.; Fox, P.F., Ed.; Springer: Boston, MA, USA, 1993; Volume 1, pp. 101–140.
31. Salaün, F.; Mietton, B.; Gaucheron, F. Buffering capacity of dairy products. *Int. Dairy J.* **2005**, *15*, 95–109. <https://doi.org/10.1016/j.idairyj.2004.06.007>.
32. Fossa, E.; Pecorari, M.; Sandri, S.; Tosi, F.; Mariani, P. The role of milk casein content in the Parmigiano-Reggiano cheese production: Chemical composition, rennet coagulation properties and dairy technological behaviour of milk. *Sci. E Tec. Latt. -Casearia* **1994**, *45*, 519–535.
33. Okigbo, L.M.; Richardson, G.H.; Brown, R.J.; Ernstrom, C.A. Variation in coagulation properties of milk from individual cows. *J. Dairy Sci.* **1985**, *68*, 822–828. [https://doi.org/10.3168/jds.S0022-0302\(85\)80899-7](https://doi.org/10.3168/jds.S0022-0302(85)80899-7).
34. Kefford, B.; Christian, M.P.; Sutherland, B.J.; Mayes, J.J.; Grainger, C. Seasonal influences on Cheddar cheese manufacture: Influence of diet quality and stage of lactation. *J. Dairy Res.* **1995**, *62*, 529–537. <https://doi.org/10.1017/S0022029900031228>.
35. Jensen, H.B.; Holland, J.W.; Poulsen, N.A.; Larsen, L.B. Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulation properties. *J. Dairy Sci.* **2012**, *95*, 2891–2903. <https://doi.org/10.3168/jds.2012-5346>.
36. Petrovska, S.; Jonkus, D.; Zagorska, J.; Ciprova, I. The influence of kappa-casein and beta-lactoglobulin genotypes on milk coagulation properties in Latvia dairy breed. *Res. Rural. Dev.* **2017**, *2*, 74–80. <https://doi.org/10.22616/rrd.23.2017.052>.

37. Cendron, F.; Franzoi, M.; Penasa, M.; De Marchi, M.; Cassandro, M. Effects of  $\beta$ - and  $\kappa$ -casein, and  $\beta$ -lactoglobulin single and composite genotypes on milk composition and milk coagulation properties of Italian Holsteins assessed by FT-MIR. *Ital. J. Anim. Sci.* **2021**, *20*, 2243–2253. <https://doi.org/10.1080/1828051X.2021.2011442>.
38. Hill, J.P.; Creamer, L.K.; Boland, M.J. Milk protein genetic polymorphism in a sample New Zealand cow population. *Livest. Prod. Sci.* **1993**, *35*, 192–193.
39. Gai, N.; Uniacke-Lowe, T.; O'Regan, J.; Faulkner, H.; Kelly, A.L. Effect of Protein Genotypes on Physicochemical Properties and Protein Functionality of Bovine Milk: A Review. *Foods* **2021**, *10*, 2409. <https://doi.org/10.3390/foods10102409>.
40. Walsh, C.D.; Guinee, T.P.; Harrington, D.; Mehra, R.; Murphy, J.J.; Connolly, J.F.; Fitzgerald, R.J. Cheddar cheesemaking and rennet coagulation characteristics of bovine milks containing  $\kappa$ -casein AA or BB genetic variants. *Milchwissenschaft* **1995**, *50*, 492–495.
41. Mayer, H.K.; Ortner, M.; Tschager, E.; Ginzinger, W. Composite milk protein phenotypes in relation to composition and cheesemaking properties of milk. *Int. Dairy J.* **1997**, *7*, 305–310. [https://doi.org/10.1016/S0958-6946\(97\)00019-8](https://doi.org/10.1016/S0958-6946(97)00019-8).
42. Lucey, J.A.; Fox, P.F. Rennet coagulation properties of late-lactation milk, Effect of pH adjustment, addition of CaCl<sub>2</sub>, variation in rennet level and blending with mid-lactation milk. *Ir. J. Agric. Food Res.* **1992**, *31*, 173–184.
43. Davis, S.R.; Farr, V.C.; Knowles, S.O.; Lee, J.; Kolver, E.S.; Auldist, M.J. Sources of variation in milk calcium content. *Aust. J. Dairy Technol.* **2001**, *56*, 156.
44. Fox, P.F.; Guinee, T.P.; Cogan, T.M.; McSweeney, P.L.H. *Fundamentals of Cheese Science*, 2nd ed.; Springer: New York, NY, USA, 2017.
45. Tyrisevä, A.M.; Vahlsten, T.; Ruottinen, O.; Ojala, M. Noncoagulation of milk in Finnish Ayrshire and Holstein-Friesian cows and effect of herds on milk coagulation ability. *J. Dairy Sci.* **2004**, *87*, 3958–3966. [https://doi.org/10.3168/jds.S0022-0302\(04\)73536-5](https://doi.org/10.3168/jds.S0022-0302(04)73536-5).
46. Chiofalo, V.; Maldonato, R.; Martin, B.; Dupont, D.; Coulon, J.B. Chemical composition and coagulation properties of Modicana and Holstein cows' milk. *Ann. De Zootech.* **2000**, *49*, 497–503. <https://doi.org/10.1051/animres:2000140>.
47. Bittante, G.; Contiero, B.; Cecchinato, A. Prolonged observation and modelling of milk coagulation, curd firming, and syneresis. *Int. Dairy J.* **2013**, *29*, 115–123. <https://doi.org/10.1016/j.idairyj.2012.10.007>.

48. Lucey, J. Cheesemaking from grass based seasonal milk and problems associated with late-lactation milk. *Int. J. Dairy Technol.* **1996**, *49*, 59–64. <https://doi.org/10.1111/j.1471-0307.1996.tb02491.x>.
49. Fantuz, F.; Polidori, F.; Cheli, F.; Baldi, A. Plasminogen activation system in goat milk and its relation with composition and coagulation properties. *J. Dairy Sci.* **2001**, *84*, 1786–1790. [https://doi.org/10.3168/jds.S0022-0302\(01\)74616-4](https://doi.org/10.3168/jds.S0022-0302(01)74616-4).
50. Bastian, E.D.; Brown, R.J.; Ernstrom, C.A. Plasmin activity and milk coagulation. *J. Dairy Sci.* **1991**, *74*, 3677–3685. [https://doi.org/10.3168/jds.S0022-0302\(91\)78557-3](https://doi.org/10.3168/jds.S0022-0302(91)78557-3).
51. Vacca, G.M.; Stocco, G.; Dettori, M.L.; Pira, E.; Bittante, G.; Pazzola, M. Milk yield, quality, and coagulation properties of 6 breeds of goats: Environmental and individual variability. *J. Dairy Sci.* **2018**, *101*, 7236–7247. <https://doi.org/10.3168/jds.2017-14111>.



## **Chapter 7**

### **General discussion**



## 7.1. Introduction

A successful dairy system depends on several qualities: efficient production, better reproduction, longevity in the herd, and farm management. Factors affecting any of these qualities will affect the farm's profitability.

Once-a-day (OAD) milking during the entire lactation has been practiced as a production system by New Zealand dairy farmers since 1990 [1], and in the 2019-2020 production season, about 8% of dairy farms were classified as OAD milking farms [2]. Once-a-day milking reduces the milk yield per cow in the range of 22-50% relative to TAD milking [3-5]. Once-a-day milking also alters the milk composition by increasing the percentages of fat and protein and decreasing the percentage of lactose compared with TAD milking [5-7]. Interestingly, OAD milking improves several aspects in terms of cow body weight, body condition score [5,8,9], and reproductive performance [4,10]. Once-a-day milking also causes less stress on farmers and creates time for additional employment or relaxation for farmers.

The major consequences of OAD milking are the reduction of milk yield and changes in milk composition [11]. Several studies have compared the effect of OAD milking on the concentrations of fat and protein as they are the main components of the New Zealand payment system. However, there have been no studies exploring the effect of OAD milking on the actual fatty acid composition of milk fat which indirectly affects the milk processing and quality of final products, for example, butter. Therefore, this study aimed to explore the fatty acid composition of milk from cows milked OAD and TAD under New Zealand grazing conditions. To achieve this aim, the following specific objectives were proposed:

1. To compare the gross composition and fatty acid composition of milk from cows milked OAD and TAD at different stages of lactation.
2. To examine the milk composition and fatty acids composition of different breeds of cows milked OAD and TAD in different stages of lactation.
3. To investigate the effect of single nucleotide polymorphism (SNP) rs109421300 on the fatty acid composition of cows milked OAD and TAD.
4. To investigate the effect of breed on the composition and coagulation properties of milk from cows milked OAD, TAD morning and TAD afternoon.

Chapter 2 provided a comprehensive systematic review of the literature available on fatty acid composition and factors affecting fatty acid composition. Chapter 2 also reviewed OAD milking and its effects on milk yield and composition. In New Zealand and overseas there have been four controlled and full-season studies exploring milk production and milk composition of OAD milk [3-5,12]. Some studies also compared the milk yield and composition between breeds [5-7] and stages of lactation [13-15] in OAD milking frequency. These studies reported mainly the milk production traits and gross milk composition (fat, protein, and lactose).

## **7.2. Comparative performance of cows milked once a day and twice a day for milk production, gross composition, and fatty acid composition**

The main research aim of this thesis was to explore the effect of milking frequency on the fatty acid composition of milk in pasture-based cows. Chapter 3 compared the milk production traits, gross composition and fatty acid composition of milk from cows milked OAD and TAD in different stages of lactation. Compared with cows milked TAD, cows milked OAD produced lower yields of milk, fat, protein and lactose, and higher percentages of fat and protein. Lower yields of milk, fat, and protein are the main concern of OAD milking although it provides several benefits. The increase in the concentrations of fat and protein (milk solids) does not compensate for the decrease in the milk yield [11]. Clark et al. [5] noted a 16.7% higher stocking rate for cows milked OAD compared with cows milked TAD to compensate for the production loss and reported a significant decrease in the production loss in OAD milking compared with TAD milking. However, an increase in the stocking rate should be practiced based on the availability of feed [11]. Overall, it seems the economic loss in OAD milking is partly compensated by the lower cost of production, additional time on better farm management, and more enjoyment of farming. It is clear that OAD milking will survive in the future as the popularity of OAD milking has started to grow among New Zealand farmers.

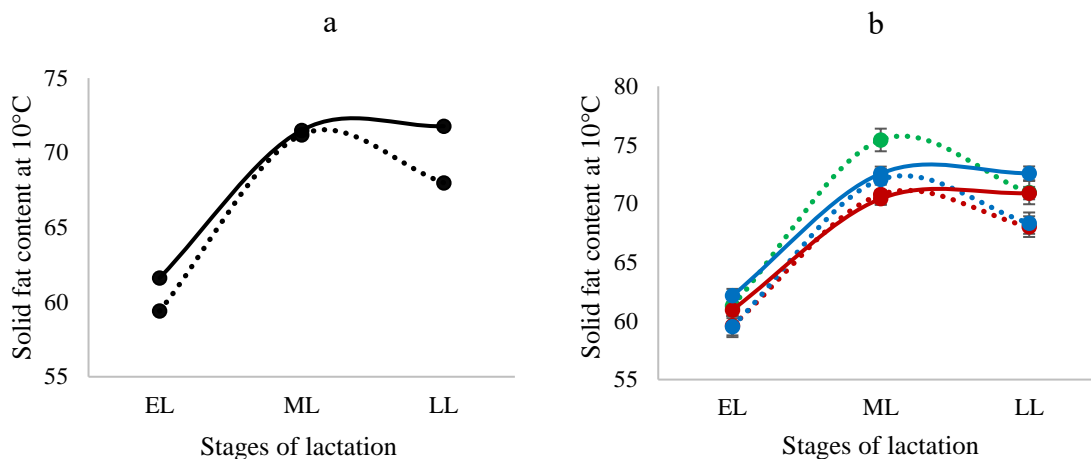
In New Zealand, the payment system for farmers is based on fat and protein yield with a penalty for milk volume. So, lower yields of fat and protein in OAD milking would lead to an economic loss. In New Zealand, OAD milking is considered a viable production system with farmers accepting the production loss but obtaining benefits of OAD milking such as labour retention, fewer labour cost, fewer management cost, better cow fertility performance, better cow health and lifestyle changes.

Chapter 3 also reports higher proportions of *de novo* synthesised fatty acids (C8:0 - C14:0) and lower proportions of long-chain fatty acids in cows milked OAD relative to cows milked TAD. Variations in these fatty acids across milking frequencies indicate that there were different mechanisms for synthesising fatty acids in cows milked OAD. Generally, fatty acids from C4:0 to half of the proportion of C16:0 (short and medium-chain fatty acids) are synthesized in the mammary gland using acetate and  $\beta$ -hydroxybutyrate as precursors by the process called *de novo* synthesis [16]. It is possible that long-time OAD milking in the selected OAD cows would have caused some changes in the uptake of precursors by the rumen, the action of enzymes involved in the fatty acid synthesis or absorption of long chain fatty acids (half of the proportion of C16:0 and all C18 fatty acids) from diet or body reserves. Massey University No.1 Dairy farm has been practicing OAD milking for more than seven seasons. Therefore, possibly the cows have adapted to OAD milking by making modifications in their synthesis of fatty acids.

The energy balance of the cows is an important factor that influences the synthesis and absorption of fatty acids. Utilization of energy is reflected in the milk fatty acid composition [17]. Higher proportions of fatty acids from C8:0 to C14:0 in cows milked OAD compared with TAD would be due to the improved energy balance in cows milked OAD. When the cow's energy balance is lower or negative, the synthesis of *de novo* synthesized fatty acids is lower and the absorption of LCFA (preformed fatty acids) from body reserves is higher [18-20]. Results from Chapter 3 showed that cows milked OAD had an improved energy balance compared with cows milked TAD due to their higher body condition score. Higher body condition scores for cows milked OAD are evidence for improved energy balance in cows [21-22]. Therefore, higher proportions of *de novo* synthesis fatty acids in cows milked OAD could be due to the improved energy balance.

Based on publications to date, stage of lactation is one of the important factors affecting fatty acid composition [18,20,23]. It was expected that the proportion of fatty acids C6:0 and C14:0 would be higher in each stage of lactation in cows milked OAD compared with cows milked TAD due to the improved energy balance. However, the proportions of these fatty acids were lower in the late lactation milk from cows milked OAD. The reason for this is not clear. The effect of the stage of lactation on fatty acid composition could be considered important as in New Zealand some dairy farmers practise OAD milking in late lactation. Higher proportions of these fatty acids could affect the processability of late lactation milk from cows milked OAD, especially the hardness of butter.

In this thesis, the solid fat content of milk was predicted using the different proportions of fatty acids. Solid fat content is the ratio between solid fat and total fat in milk. Generally, solid fat content at 10°C (SFC<sub>10</sub>) is used to compare the properties of milk fat, especially butter hardness [24]. Solid fat content at 10°C is a useful property that is strongly related to the individual fatty acids in milk [24,25] and their arrangements in triglycerides [26]. An increase in the proportions of C16:0 and a decrease in the proportion of C18:1 *cis* 9 in milk will increase the SFC<sub>10</sub> of milk [27]. Higher SFC<sub>10</sub> would result in lower spreadability in butter. In this study, milk from OAD milking could produce a more easily spreadable butter compared with milk from TAD milking as the proportions of C16:0 and C18:0 and predicted SFC<sub>10</sub> was lower compared with cows milked TAD throughout the lactation. When comparing stages of lactation, SFC<sub>10</sub> was lower in early lactation (Figure 7.1a) and this is in agreement with Auld et al. [28] and Thomson and Van-der-Pol [29].



**Figure 7.1.** Proportions of (a) predicted solid fat content at 10°C of milk from cows milked once a day (OAD) (....) and twice a day (TAD) (—) and (b) predicted solid fat content at 10°C of milk from Holstein-Friesian (.....), Holstein-Friesian × Jersey (.....), and Jersey (.....) cows milked OAD and Holstein-Friesian (—) and Holstein-Friesian × Jersey (—) cows milked TAD during the 2020-2021 production season.

EL = early lactation (<90 days); ML = mid-lactation (90-180 days); LL = late lactation (>180 days). The vertical bars show standard errors.

Lucey [30] reported in countries that have seasonal milk production systems, for example, New Zealand, it would be difficult to produce good quality cheese from late lactation milk due to poor milk coagulation properties of milk compared with other stages of lactation. Interestingly, when considering producing butter, the SFC<sub>10</sub> was lower in OAD milking during late lactation compared with TAD milking. This suggests that there is a

possible solution to produce good quality (more easily spreadable butter) in late lactation using OAD milk. Therefore, OAD milking will be an easy and cost-effective way to produce softer milk fat and ultimately softer butter rather than other factory techniques, for example, fractionation.

One of the other factors affecting fatty acid composition is breed. The effect of breed and the interaction between breed and stages of lactation within the milking frequencies were studied in Chapter 4. Breeds performed differently in each stage of lactation in different milking frequencies. Holstein-Friesian (F) cows milked OAD produced a lower proportion of C18:0 compared with Jersey (J) cows milked OAD in early and mid-lactation. Changes occurred in mid- and late lactation as the SFC<sub>10</sub> of J cows milked OAD was higher compared with F and Holstein-Friesian × Jersey (F×J) cows milked OAD (Figure 7.1b), suggesting that J milk would be less suitable to produce more easily spreadable butter. The results suggest the effect of the breed on fatty acid composition and SFC<sub>10</sub> was less compared with the effect of stages of lactation. When considering breed regardless of stages of lactation, the only fatty acid that varied between F and J was C18:0. Holstein-Friesian cows milked OAD produced a lower proportion of C18:0 compared with J cows milked OAD. Similarly, the predicted SFC<sub>10</sub> was lower for F cows compared with J cows. On the other hand, C18:0 was the only fatty acid that varied significantly between F and J cows milked TAD. However, previous studies, exploring the effect of breed on the fatty acid composition of cows milked TAD, reported significant differences between several individual fatty acids [24,31]. The difference in the results could be due to the variation in feed, management, sample size, and analytical method followed.

Previous studies reported that J cows are more suitable for OAD milking as their production loss is smaller compared with F cows [5-7]. However, in terms of fatty acid when selecting J cows for OAD milking, they would produce higher C18:0 and higher SFC<sub>10</sub>, which could lead to lower spreadability of butter compared with F cows. The findings of this thesis suggest F×J cows performed intermediate for most fatty acids and SFC<sub>10</sub> compared with F and J. It is well-known that F cows are superior for milk yield traits compared with J cows while J cows are superior for milk solids compared with F cows. Holstein-Friesian × Jersey cows are preferred for higher farm profitability and heterosis effect for milk production traits. Recently, the percentage of F×J cows in the New Zealand national herd has increased. In 2020/2021 the national herd consisted of

49.6% F×J, 32.5% F and 8.2% J cows [32]. For the current payment system, it is suitable to include more F×J, higher fat and protein yield with the penalty of milk volume. However, F×J cows are more likely to produce more easily spreadable butter compared with J cows but the spreadability would more likely to be lower compared to F cows.

The findings of this study will inform the dairy industry about the fatty acid composition of milk especially, OAD milk. The information is also valuable for milk producers and processing companies to improve the quality of milk and dairy products. Fatty acids in milk are considered important by consumers in terms of health. The findings of this thesis will have practical implications for farmers who practice OAD milking or planning to switch to OAD milking. The fatty acid composition and SFC<sub>10</sub> of milk from cows milked OAD suggests that cows milked OAD could provide milk with more soft fat which would lead to better processing properties especially making more spreadable butter. The results of this study also could help researchers, farmers, and processing companies to think of a possible way to produce a more easily spread butter with OAD milking. This thesis reports that OAD farmers have been producing milk with softer milkfat which could obtain a premium if milk fatty acid composition becomes more valuable to consumers in the future. It is also clear that including F cows in the OAD herd could result in milk that is more suitable to produce more easily spreadable butter compared with J cows.

### **7.3. Genetic variability for fatty acids**

The objective of Chapter 5 was to investigate the effect of SNP rs109421300 of the DGAT1 gene on chromosome 14 on the fatty acid composition of milk. Chapter 5 demonstrated that the CC genotype for this SNP was associated with higher fat yield, fat percentage, protein percentage, and lower milk yield and protein yield. The CC genotype was also associated with higher proportions of C16:0 and C18:0 and predicted SFC<sub>10</sub>, as well as lower proportions of C4:0 and C18:1 *cis* 9 in both milking frequencies. However, the magnitude of expression of the CC genotype was different between milking frequencies for some milk components which is reflective of a genotype by environment interaction. The CC genotype in cows milked OAD produced higher percentages of fat and protein and lower proportions of C16:0, and C18:0 and predicted SFC<sub>10</sub> compared with the CC genotype in cows milked TAD. The genetic and environmental interaction causes variations in a genotype of a trait when exposed to different environments [33], this effect is clear in this study.

These results would be helpful for the New Zealand dairy industry and breeding companies when selecting cows for breeding programmes. Selecting cows for the CC genotype of SNP rs109421300 would be beneficial for the New Zealand dairy industry as they produce higher fat yield, fat percentage and protein percentage. A similar suggestion was given by Lopez-Villalobos et al. [34] based on the production traits. But when considering processability, especially butter making, the CC genotype would lead to less spreadable butter compared with the TT genotype in both milking frequencies. Similar results would be expected when selecting the CC genotypes in cows milked OAD. However, a specific breeding programme for OAD milking has not yet been developed.

Although there has been no specific breeding programme for OAD milking, New Zealand has a successful breeding programme for TAD milking. In New Zealand and other countries, breeding programme is developed considering mainly fat, protein and milk yield. But recent studies focused more on the genetic basis of fatty acids and some studies also suggested including fatty acid composition in the breeding programme [35,36]. Genetic evaluation of fatty acids showed low to moderate heritability for most of the fatty acids [35-38]. As in other main milk components, the estimation of genetic parameters such as the estimation of breeding value led to genomic selection approaches in fatty acid. Already GWAS for the fatty acids composition of milk has been undertaken to identify the genomic regions responsible for individual fatty acids using SNPs [39-41]. Similarly, the estimation of genomic breeding values for fatty acids has become an important breakthrough to perform genomic selection. Cesarani et al. [37] reported higher accuracy for genomic breeding values compared with the traditional pedigree-based breeding value in sheep milk fatty acid composition. Feritas et al. [38] reported moderate to high accuracy for genomic breeding values could be obtained using the genomic best linear unbiased prediction method. These discoveries suggest the possibilities for considering milk fatty acids as an important trait to incorporate in the breeding programme.

Estimation of heritability, genetic correlation and genomic breeding values for composition and processability traits shows the possibilities for genetic improvement and genomic selection for processability. Ikonen et al. [42], Cassandro et al. [43] and Sanchez et al. [44] reported moderate to strong heritability estimates of 0.2-0.25 for rennet coagulation time and 0.15-0.4 for curd firmness at 30 minutes. Cassandro et al. [43] reported positive genetic correlations between curd firmness and protein percentage (0.44) and casein content (0.53). These parameters could help in the process of genomic

selection for cheese-making properties. Glantz et al. [45] reported direct genomic values for milk composition and coagulation properties. They reported cheese yield was negatively correlated with direct genomic breeding value for milk and positively correlated with direct genomic value for fat percentage and direct genomic value of protein percentage. The study also suggested the estimation of direct genomic values of production traits, composition traits, and processability traits will help to predict the composition and cheese-making properties of milk in the early life of animals based on their genome. Based on the above evidence, successful genomic selection for production traits, MCPs, and processability traits would be possible when studies are conducted with larger population sizes and more SNP markers. Up to now, there have been no studies evaluating the genetic parameters and genomic selection for SFC<sub>10</sub>, hardness or spreadability.

Overall, this study suggests that including fatty acid composition in the selection index and breeding programme could be beneficial for countries like New Zealand as it is one of the major dairy exporters in the world. In the long term, selecting OAD cows, especially with the TT genotype of SNP rs109421300 can be considered only for butter making if the consumer preference for more easily spreadable butter increases.

#### **7.4. Milk composition and processability**

Milk composition is one of the factors affecting the quality and yield of final products. In this thesis, the processability of milk has been discussed, mainly the processability of milk into butter. The importance of fatty acids in making butter has been discussed using SFC<sub>10</sub>, an index that was developed using the proportions of individual fatty acids.

Chapter 3 reported that cows milked OAD produced milk with softer fat, which could result in more easily spreadable butter, especially in early lactation. Chapter 4 concluded that milk from F cows milked OAD would be more likely to produce more easily spreadable butter than F×J and J cows, particularly in early lactation. Chapter 5 has reported the TT genotype of SNP rs109421300 would produce more easily spreadable butter compared with the CC genotype. This suggests the fatty acid composition of milk determines the spreadability of butter by affecting the SCF<sub>10</sub> of milk.

Chapter 6 explored the effect of breeds on milk composition and milk coagulation properties (MCP) of cows milked OAD and TAD at different milking times. The milk coagulation properties provide a means of determining the acceptable milk for cheese

making. Jersey cows were superior for milk fat and protein percentages and MCPs compared with F cows. Holstein-Friesian  $\times$  Jersey cows were intermediate for milk composition and MCPs in each milking frequency-milking time. However, the correlations between milk composition and MCPs were weak to moderate. These results suggest MCPs were affected by a combination of factors or more complex factors. The correlations between individual fatty acids and MCPs were not significant in this study.

The relationship between milk composition and MCPs depended on milking frequency-milking time. In OAD milk, MCPs did not correlate with percentages of fat and protein, in TAD morning milking MCPs were only correlated with the percentage of fat, and in TAD afternoon milk correlated with both fat and protein percentages. These results suggest correlations were affected by milking frequency-milking time and correlations were not constant to predict the MCPs. Auld et al. [31] reported that the MCPs were controlled by a combination of several complex factors such as breed, the genetic variability of different casein fractions and  $\beta$ -lactoglobulin, calcium concentration of milk, pH, and temperature. In Chapter 6, greater MCPs for J cows were indirectly due to the composition. Auld et al. [31] also reported better MCPs in J cows compared with F cows and the reason could be the superiority of J cows for milk fat and protein percentages. Genetic variation for other factors affecting MCPs also should be considered when exploring the breed effect. One of the other factors affecting MCPs is the genetic variation of  $\beta$ -lactoglobulin. The BB variant is assumed to be related to variation in protein structure, a higher ratio of  $\kappa$ -casein to other casein fractions, and higher casein to whey ratio in milk compared with AA or AB variants [46].

When considering practical applications of the results in processing, in OAD milking, selecting milk from J cows would be suitable for cheese making due to higher MCPs. However, the findings of Chapter 4 and SFC<sub>10</sub> of different breeds suggest selecting J cows milked OAD would tend to reduce the spreadability of butter. Several studies have reported that J cows were more suitable for OAD milking. According to the results of this study, when selecting J cows, the milk would be more suitable for cheese making. If OAD farmers include more F cows in their herd, then the milk would be more suitable for butter making. Selecting F $\times$ J cows could result in intermediate values for MCPs and butter spreadability. For this to happen dairy companies need to implement a payment system that rewards these traits, for instance, the proportions of unsaturated fatty acids (UFA) [47] or SCF<sub>10</sub> or an index developed by incorporating the three MCPs studied in Chapter

6. This could be initiated by calculating the breeding values of UFA especially, C18 fatty acids, processability parameters, and products to show farmers what the cows and bulls are suitable for in terms of developing dairy products. The next step could also be done by incorporating these traits in the selection index. Initiation of this payment system would be helpful for OAD farmers as based on the results of this thesis, cows milked OAD are more likely to produce milk that could result in more easily spreadable butter and good quality cheese. However, developing and implementing such a payment system would be a long-term process and would depend on the market demand for the products.

In terms of the findings of this thesis, OAD milking reduced production compared with TAD milking. However, the fatty acid composition and  $SFC_{10}$  of OAD milking suggest that OAD milk would be more suitable when making more easily spreadable butter. In the future, when consumer preference for milk with particular fatty acid composition increases, OAD milking would be a suitable option in New Zealand due to its preferable fatty acid composition. Moreover, this research was conducted on Massey University No. 1 Dairy farm where the cows have been successfully milked OAD for more than seven seasons. The farm has found several benefits in production and management [48]. One of the areas highly studied in New Zealand recently is the contribution of the dairy industry to methane emissions. Methane emission is positively correlated with fatty acids from C4:0 to C16: [49-51]. The findings of this thesis report that cows milked OAD produced higher proportions of fatty acids from C8:0 to C14:0. This could result in higher methane emissions. However, these results would be more accurate if other components such as dietary intake, milk yield, milk composition, and milk fatty acids composition are used in the prediction of methane emission.

### **7.5. Limitation of this thesis**

The analysis undertaken in this thesis was conducted under On-farm conditions. Massey University farms are well-established, and it was not possible to completely design the experiment, manage the animals and control the feed. Therefore, the outcome of the research could be affected by uncontrolled effects.

As the analysis was conducted in real-farm conditions, the effect of feed was confounded with milking frequency. Feed is one of the main factors affecting the fatty acid composition of milk [52]. As the feed provided for the OAD herd was slightly different from the feed provided for the TAD herd, the conclusions of this thesis must be handled

with caution. In theory, fatty acids from C4:0 to C14:0 and half of the proportion of C16:0 are produced by *de novo* synthesis and the remaining proportion of C16:0 and C18:0 fatty acids (preformed fatty acids) are absorbed from the feed or body reserve of cows [16]. Generally, cows that are fed diets containing some fatty acids would also affect the proportion of *de novo* fatty acids in milk [53,54]. For example, in New Zealand, some processing companies limit feeding palm kernel expeller as a supplementary feed, as feeding palm kernel expeller affects the fatty acid composition of milk, especially the proportion of C12:0 [55] which could affect the processibility of milk. Therefore, in this study, the feed provided in both farms could have an effect on the fatty acid composition of milk. The impossibility of allocating the same supplementation level in both OAD and TAD herds was considered a confounding effect, which affected the statistical analysis to separate the effect of milking frequency and feed. On the other hand, a low supplementation level in the OAD herd and higher supplementation in the TAD herd would be acceptable as cows milked OAD have lower dry matter requirements compared with cows milked TAD [3,56].

In this thesis, a comparison between breed performance for fatty acid composition across milking frequencies did not perform as the TAD herd lacked J cows (Chapter 4 and Chapter 6). This has also created challenges in making a proper experimental design.

Future experiments can be conducted using the following features of the experimental design. Important factors that should be considered are the sample size, breed composition, parity of the cows, stages of lactation, and season of the year. A power analysis was conducted using the data obtained in this study. The following assumptions were made: the samples were selected randomly and were independent of each other, the dependent variable followed a normal distribution, and the true mean and standard deviation were known and not estimated. According to the power analysis, 30 cows can be allocated to OAD and TAD herds randomly. Breed composition representing the New Zealand national herd (49.1% F×J, 32.7% F and 8.4% J) would give comparable results. The parity of the cows can be selected based on the replacement rate (22%) with primiparous and multiparous cows. Auld et al. [28] reported the effect of season and stages of lactation on milk composition using a proper experimental design. Selected cows can be divided into four groups and allowed to calve in the interval of three months with one herd calving each of January, April, July, and October. These cows can be milked four times during the production season (September, December, March, and

June). Each cow could be sampled from on three occasions (early, mid-, and late lactation). This will ensure every three stages of lactation during every season (spring in September, summer in December, autumn in March, and winter in June).

In this study calibration equations were developed using the partial least square method to predict the fatty acid composition. There are also other prediction models such as Bayesian or alternative machine learning approaches [57]. The accuracy of the calibration equations would be higher if the number of samples was higher. This study developed the calibration equations using the samples from early lactation. The accuracy could have been improved if some samples from mid- and late lactation were included. Tiplady et al. [57] reported that increasing the number of samples and including samples from different herds would increase the accuracy of the calibration equations. Using samples from different regions and different feeding regimes would also increase the accuracy of the calibration equations. Most of the fatty acids in milk are present in small proportions. Therefore, increasing the sample numbers would increase the accuracy of prediction.

Attempts to develop calibration equations for predicting MCPs were limited for the small sample size. Few studies have developed calibration equations to predict the MCPs of milk. The coefficient of determination value for RCT was 0.62 [58] and 0.76, 0.72 and 0.7 for RCT, K<sub>20</sub> and A<sub>30</sub>, respectively [59]. De Marchi et al. [60] reported that the prediction accuracy of the equations was satisfactory and could be used in the payment system to give a premium or penalty for MCPs. This could also be considered in the New Zealand payment system as the normal herd test has been performed using mid-infrared spectroscopy and including the MCP calibration equations would be useful in terms of both milk and milk product profit. Farmers could be paid for milk solid and MCPs. These equations can be used to predict MCP values for noncoagulating samples within 30 minutes [60]. In this thesis, non-coagulating samples were reported as this is common using a traditional Formagraph within 30 minutes. Therefore, the development of calibration equations for MCP would be beneficial for the New Zealand dairy industry, maybe in the future.

Apart from these limitations, the findings of this thesis provide useful insights for knowing the fatty acids composition of OAD milk and the effect of OAD milk on processability.

## 7.6. Future research

Some findings of this thesis may lead to future research projects. The findings would give information for dairy farmers, researchers, processing companies, breeding companies, and consumers about fatty acids in milk, especially in OAD milk. The mechanism of *de novo* synthesis is already known in cows. Delamaire and Guinard-Flament [61] studied the mechanism of *de novo* synthesis and uptake of precursors into the mammary gland in cows milked in different intervals for a short period. Further research on the mechanism of *de novo* synthesis and absorption should be attempted in cows milked OAD over several years.

Chapter 5 only examined one SNP in chromosome 14 of cows milked OAD and TAD. However, several studies reported that SNPs on chromosomes 5, 19 and 26 regions have a major effect on fatty acid composition in cows milked TAD [62-64]. Future studies should be carried out by performing GWAS analysis to identify chromosomes and SNPs associated with fatty acid composition in cows milked OAD. Future studies also can be planned with a large number of samples to get more accurate results.

Milk coagulation properties are affected by a combination of several factors [31]. In Chapter 6, mainly the effect of breed on MCPs was studied with milk fat and protein percentages. Future studies can be conducted to explore the effect of proportions of different protein fractions, especially  $\kappa$ -casein and its genetic variants, and the concentration of calcium ions on MCPs [46] in cows milked OAD. Milk coagulation properties studied in this thesis are important factors to determine the quality and yield of cheese [65]. It is recommended that making cheese at a representative scale from milk from cows milked OAD and comparing the cheese yield, moisture content, and nutrient recovery would be more useful to the New Zealand dairy industry [66].

In Chapters 3, 4, and 5, the  $SFC_{10}$  of milk was predicted using an index developed with fatty acids. The information provided on  $SCF_{10}$  would be useful and can be considered as a foundation for conducting future studies on actual  $SCF_{10}$  of milk. Solid fat content analysis is carried out by the nuclear magnetic resonance technique [24,26]. Future studies can be also attempted to develop butter from OAD milk and study the physical properties of butter, especially the spreadability of butter. MacGibbon [24] reported milk entering the factory has variations in milk fat properties. Therefore, further studies on the

characteristics and processability of milk from cows milked OAD should be carried out to identify suitability for processing.

With all the above suggestions, a future experiment can be conducted using the suggested features of the experimental design.

### **7.7. General conclusions**

The results of this study reveal that milking cows OAD would be advantageous due to its fatty acid composition which may be suitable in terms of processability. The proportions of *de novo* synthesis fatty acid were higher, and LCFA were lower in cows milked OAD than in cows milked TAD. The proportions of C8:0 and C14:0 were lower in the late lactation milk from cows milked OAD compared with the late lactation milk from cows milked TAD. The lower proportion of long-chain saturated fatty acids and lower predicted SFC<sub>10</sub> of milk from cows milked OAD suggest that OAD milk could produce more easily spreadable butter.

Holstein-Friesian cows produced lower proportions of C18:0 in both milking frequencies with F cows milked OAD producing lower proportions, especially in early and mid-lactation, and F cows milked TAD producing a lower proportion in early and late-lactation. Holstein-Friesian cows are more likely to produce softer butter than other breeds of cows regardless of the milking frequency.

Genetic selection of cows with the CC genotype of SNP rs109421300 could be beneficial for the New Zealand dairy industry with the current payment system. However, cows with the CC genotype would be less suitable for making more easily spreadable butter due to higher predicted SCF<sub>10</sub>. Selecting cows superior for milk processability is not yet fully practiced in New Zealand as the priority is given to the yields of fat and protein with a penalty for milk volume. This could be considered in the future if the consumer/processor preference for specific fatty acid composition becomes greater.

Selecting J cows would result in superior MCPs which are suitable for cheese-making in each milking frequency-sampling time. However, the suitability of the breed for cheese-making is not only decided by milk composition but also by other several other factors.

Overall, OAD milking modifies the milk fatty acids composition therefore the processability of milk, mainly the production of butter. Cows milked OAD are more likely to produce more easily spreadable butter compared with cows milked TAD. It is known

that OAD farmers have been producing milk with softer fat which is more suitable for producing more easily spreadable butter. There are significant differences between cows milked OAD and TAD with regards to fatty acid composition and, therefore, the processability. Fatty acid composition could be included in the payment system if milk consumers are willing to pay a premium for products with desired characteristics. The inclusion of fatty acids in the payment system would not be a difficult task for New Zealand processing companies as the milk fatty acid composition is tested as a routine practice in the herd test.

## References

1. Davis, S.R.; Farr, V.C.; Stelwagen, K. Regulation of yield loss and milk composition during once-daily milking: A review. *Livest. Prod. Sci.* **1999**, *59*, 77–94, [https://doi.org/10.1016/S0301-6226\(98\)00204-8](https://doi.org/10.1016/S0301-6226(98)00204-8)
2. DairyNZ. 2020. Milking intervals. <https://www.dairynz.co.nz/milking/milking-intervals/> Accessed on 17th March 2023.
3. Holmes, C.W.; Wilson, G.F.; Mackenzie, D.D.; Purchas, J. The effects of milking once-daily throughout lactation on the performance of dairy cows grazing on pasture. *Proc. N. Z. Soc. Anim. Prod.* **1992**, *52*, 13-16.
4. Rémond, B.; Pomiès, D.; Dupont, D.; Chilliard, Y. Once-a-day milking of multiparous Holstein cows throughout the entire lactation: Milk yield and composition, and nutritional status. *Anim. Res.* **2004**, *53*, 201–212, <https://doi.org/10.1051/animres:2004014>
5. Clark, D.A.; Phyn, C.V.C.; Tong, M.J.; Collis, S.J.; Dalley, D.E. A systems comparison of once-versus twice daily milking of pastured dairy cows. *J. Dairy Sci.* **2006**, *89*, 1854–1862. [https://doi.org/10.3168/jds.S0022-0302\(06\)72254-8](https://doi.org/10.3168/jds.S0022-0302(06)72254-8)
6. Tong, M.J.; Clark, D.A.; Cooper, C.V. Once-a-day milking: Possible and profitable? *Proc. N. Z. Grassl. Assoc.* **2002**, *64*, 33–37. <https://doi.org/10.33584/jnzg.2002.64.2470>
7. Lembeye, F.; López-Villalobos, N.; Burke, J.L.; Davis, S.R. Estimation of breed and heterosis effects for milk traits and somatic cell scores in cows milked once and twice daily in New Zealand. *Proc. N. Z. Soc. Anim. Prod.* **2015**, *75*, 60–63.
8. Rémond, B.; Aubailly, S.; Chilliard, Y.; Dupont, D.; Pomiès, D.; Petit, M. Combined effects of once-daily milking and feeding level in the first three weeks of lactation

- on milk production and enzyme activities, and nutritional status, in Holstein cows. *Anim. Res.* **2002**, *51*, 101–117. <https://doi.org/10.1051/animres:2002014>
9. Phyn, C.V.C.; Kay, J.K.; Rius, A.G.; Morgan, S.R.; Roach, C.S.; Grala, T.M.; Roche, J.R. Effect of temporary alterations to milking frequency during the early post-partum period on milk production and body condition score in grazing dairy cows. *Proc. N. Z. Soc. Anim. Prod.* **2011**, *71*, 45–49.
  10. O'Brien, B.; Ryan, G.; Meaney, W.J.; McDonagh, D.; Kelly, A. Effect of frequency of milking on yield, composition, and processing quality of milk. *J. Dairy Res.* **2002**, *69*, 367–374. <https://doi.org/10.1017/S0022029902005605>
  11. Stelwagen, K.; Phyn, C.V.V.; Davis, S.R.; Guinard-Flament, J.; Pomiès, D.; Roche, J.R.; Kay, J.K. Invited review: Reduced milking frequency: Milk production and management implications. *J. Dairy Sci.* **2013**, *96*, 3401–3413. <https://doi.org/10.3168/jds.2012-6074>.
  12. Claesson, O.; Hansson, A.; Gustafsson, N.; Brannang, E. Studies on monozygous cattle twins. XVII. Once-a-day milking compared with twice-a-day milking. *Acta Agriculturae Scandinavica B.* **1959**, *9*, 38–58. <https://doi.org/10.1080/00015125909433307>
  13. Stelwagen, K.; Knight, C.H. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. *J. Dairy Sci.* **1997**, *64*, 487–494, <https://doi.org/10.1017/s0022029997002458>
  14. Davis, S.; McNaughton, L.; Bracefield, G.; Sanders, K.; Spelman, R. Variation in milk yield response to once-daily milking in Friesian-Jersey crossbred cattle. *J. Dairy Sci.* **2006**, *89*(Suppl. 1), 146–147. (Abstr.)
  15. Kay, J.K.; Phyn, C.V.; Rius, A.G.; Morgan, S.R.; Grala, T.M.; Roche, J.R. Once-daily milking during a feed deficit improves energy status in early lactating dairy cows. *Proc. N. Z. Soc. Anim. Prod.* **2012**, *72*, 129–133.
  16. MacGibbon, A.K.H. Composition and Structure of Bovine Milk Lipids. In *Advanced Dairy Chemistry*, 4th ed.; McSweeney, P.L.H., Fox, P.F., O'Mahony, J.A., Eds.; Springer: New York, NY, USA, 2020; Volume 2, pp. 1–32.
  17. Vranković, L.; Aladrović, J.; Octenjask, D.; Bijelić, D.; Cvetnić, L.; Stojević, Z. Milk fatty acid composition as an indicator of energy status in Holstein dairy cows. *Arch. Anim. Breed.* **2017**, *60*, 205–212, <https://doi.org/10.5194/aab-60-205-2017>

18. Stoop, W.M.; Bovenhuis, H.; Heck, J.M.L.; van Arendonk, J.A.M. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *J. Dairy Sci.* **2009**, *92*, 1469–1478, <https://doi.org/10.3168/jds.2008-1468>
19. Bastin, C.; Gengler, N.; Soyeurt, H. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. *J. Dairy Sci.* **2011**, *94*, 4152–4163. <https://doi.org/10.3168/jds.2010-4108>
20. Gross, J.; van Dorland, H.A.; Bruckmaier, R.M.; Schwarz, F.J. Milk fatty acid profile related to energy balance in dairy cows. *J. Dairy Sci.* **2011**, *78*, 479–488, <https://doi.org/10.1017/S0022029911000550>
21. Rémond, B.; Pomiès, D. Once-daily milking of dairy cows: A review of recent French experiments. *Anim. Res.* **2005**, *54*, 427–442, <https://doi.org/10.1051/animres:2005040>
22. Patton, J.; Kenney, D.A.; Mee, J.F.; O'Mara, F.P.; Wathes, DC.; Cook, M.; Murphy, J.J. Effect of milking frequency and diet on milk production, energy balance, and reproduction in dairy cows. *J. Dairy Sci.* **2006**, *89*, 1478–1487. [https://doi.org/10.3168/jds.S0022-0302\(06\)72215-9](https://doi.org/10.3168/jds.S0022-0302(06)72215-9)
23. Kgwatalala, P.M.; Ibeagha-Awemu, E.M.; Mustafa, A.F.; Zhao, X. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. *Anim. Genet.* **2009**, *40*, 609–615.
24. MacGibbon, A.K. Herd-to-herd variations in the properties of milkfat. *Proc. N. Z. Soc. Anim. Prod.* **1996**, *56*, 224–227.
25. Mackle, T.R.; Petch, S.F.; Bryant, A.M.; Auldist, M.J.; Henderson, H.V.; MacGibbon, A.K.H. Variation in the characteristics of milkfat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *N. Z. J. Agric. Res.* **1997**, *40*, 349–359, <https://doi.org/10.1080/00288233.1997.9513254>
26. Pacheco-Pappenheim, S.; Yener, S.; Goselink, R.; Quintanilla-Carvajal, M.X.; van Valenberg, H.J.; Hettinga, K. Bovine milk fatty acid and triacylglycerol composition and structure differ between early and late lactation influencing milk fat solid fat content. *Int. Dairy J.* **2022**, *131*, 1–9. <https://doi.org/10.1016/j.idairyj.2022.105370>
27. MacGibbon AKH. 1994. Relationship between SFC and the fatty acid composition of milkfat. Brief Communication of the 24th International Dairy Congress, Melbourne, 18–22 September 1994. Australian National Committee of the International Dairy Federation, Glen Iris, Victoria, Australia

28. Auldist, M.J.; Walsh, B.J.; Thomson, N.A. Seasonal and lactational influences on bovine milk composition in New Zealand. *J. Dairy Res.* **1998**, *65*, 401–411, <https://doi.org/10.1017/s0022029998002970>
29. Thomson, N.A.; Van-der-Pol, W. Seasonal variation of the fatty acid composition of milkfat from Friesian cows grazing pasture. *Proc. N. Z. Soc. Anim. Prod.* **2000**, *60*, 314-317.
30. Lucey, J. Cheesemaking from grass based seasonal milk and problems associated with late-lactation milk. *Int J Dairy Technol.* **1996**, *49*, 59-64.
31. Auldist, M.J.; Johnston, K.A.; White, N.J.; Fitzsimons, W.P.; Boland, M.J. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *J. Dairy Sci.* **2004**, *71*, 51–57, <https://doi.org/10.1017/s0022029903006575>
32. LIC and DairyNZ. 2021. New Zealand Dairy Statistics 2020-21. <https://www.dairynz.co.nz/media/5794941/nz-dairy-statistics-2020-21-web.pdf>  
Accessed on 21<sup>th</sup> December 2022.
33. Ottman, R. Gene-environment interaction: definitions and study design. *Prev. Med.* **1996**, *25*, 764-770. <https://doi.org/10.1006/pmed.1996.0117>
34. Lopez-Villalobos, N.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Holroyd, S.E.; MacGibbon, A.K.; Snell, R.G. Estimation of genetic and crossbreeding parameters of fatty acid concentrations in milk fat predicted by mid-infrared spectroscopy in New Zealand dairy cattle. *J. Dairy Sci.* **2014**, *81*, 340–349. <https://doi.org/10.1017/S0022029914000272>
35. Stoop, W.M.; Van Arendonk, J.A.; Heck, J.M.; Van Valenberg, H.J.; Bovenhuis, H. Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians. *J. Dairy Sci.* **2008**, *91*, 385-394. <https://doi.org/10.3168/jds.2007-0181>
36. Correddu, F.; Cellesi, M.; Serdino, J.; Manca, M.G.; Contu, M.; Dimauro, C.; Ibba, I.; Macciotta, N.P. Genetic parameters of milk fatty acid profile in sheep: Comparison between gas chromatographic measurements and Fourier-transform IR spectroscopy predictions. *Animal.* **2018**, *13*, 469-476. <https://doi.org/10.1017/S1751731118001659>
37. Cesarani, A.; Gaspa, G.; Correddu, F.; Cellesi, M.; Dimauro, C.; Macciotta, N.P. Genomic selection of milk fatty acid composition in Sarda dairy sheep: Effect of

- different phenotypes and relationship matrices on heritability and breeding value accuracy. *J. Dairy Sci.* **2019**, *102*, 3189-203. <https://doi.org/10.3168/jds.2018-15333>
38. Freitas, P.H.; Oliveira, H.R.; Silva, F.F.; Fleming, A.; Miglior, F.; Schenkel, F.S.; Brito, L.F. Genomic analyses for predicted milk fatty acid composition throughout lactation in North American Holstein cattle. *J. Dairy Sci.* **2020**, *103*, 6318-6331. <https://doi.org/10.3168/jds.2019-17628>
39. Bouwman, A.C.; Visker, M.H.P.W.; van Arendonk, J.M.; Bovenhuis, H. Genomic regions associated with bovine milk fatty acids in both summer and winter milk samples. *BMC Genet.* **2012**, *13*, 1-13.z
40. Olsen, H.G.; Knutsen, T.M.; Kohler, A.; Svendsen, M.; Gidskehaug, L.; Grove, H.; Nome, T.; Sodeland, M.; Sundsaasen, K.K.; Kent, M.P.; Martens, H. Genome-wide association mapping for milk fat composition and fine mapping of a QTL for *de novo* synthesis of milk fatty acids on bovine chromosome 13. *Genet. Sel.* **2017**, *49*, 1-3. <https://doi.org/10.1186/s12711-017-0294-5>
41. Palombo, V.; Milanese, M.; Sgorlon, S.; Capomaccio, S.; Mele, M.; Nicolazzi, E.; Ajmone-Marsan, P.; Pilla, F.; Stefanon, B.; D'Andrea, M. Genome-wide association study of milk fatty acid composition in Italian Simmental and Italian Holstein cows using single nucleotide polymorphism arrays. *J. Dairy Sci.* **2018**, *101*, 11004-19. <https://doi.org/10.3168/jds.2018-14413>
42. Ikonen, T.; Morri, S.; Tyrisevä, A.M.; Ruottinen, O.; Ojala, M. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. *J. Dairy Sci.* **2004**, *87*, 458-467. [https://doi.org/10.3168/jds.S0022-0302\(04\)73185-9](https://doi.org/10.3168/jds.S0022-0302(04)73185-9)
43. Cassandro, M.; Comin, A.; Ojala, M.; Dal Zotto, R.; De Marchi, M.; Gallo, L.; Carnier, P.; Bittante, G. Genetic parameters of milk coagulation properties and their relationships with milk yield and quality traits in Italian Holstein cows. *J. Dairy Sci.* **2008**, *91*, 371-76. <https://doi.org/10.3168/jds.2007-0308>
44. Sanchez, M.P.; Tribout, T.; Fritz, S.; Wolf, V.; Laithier, C.; Brochard, M.; Boichard, D. Opportunities for genomic selection of cheese-making traits in Montbéliarde cows. *J. Dairy Sci.* **2022**, *105*, 5206-5220. <https://doi.org/10.3168/jds.2021-21558>
45. Glantz, M.; Månsson, H.L.; Paulsson, M.; Stålhammar, H. Genomic selection in relation to bovine milk composition and processability. *J. Dairy Res.* **2012**, *79*, 53-59. <https://doi.org/10.1017/S002202991100077X>

46. Amenu, B.; Deeth, H.C. The impact of milk composition on cheddar cheese manufacture. *Aust. J. Dairy Technol.* **2007**, *62*, 171–184.
47. Silva-Villacorta, D.; Lopez-Villalobos, N.; Blair, H.T.; Hickson, R.E.; Spelman, R.J.; Melis, J.; Davis, S.R.; Berry, S.D.; Lehnert, K.; Snell, R.G. Effect of lactation stage and breed on the concentration of unsaturated fatty acids in milkfat of New Zealand dairy cattle. *Proc. N. Z. Soc. Anim. Prod.* **2011**, *71*, 203-207.
48. DairyNZ. 2017. Full season once-a-day (OAD) milking. <https://www.dairynz.co.nz/milking/milking-intervals/once-a-day-milking/full-season-once-a-day-oad-milking/> Accessed on 14th December 2022.
49. Chilliard, Y.; Martin, C.; Rouel, J.; Doreau, M. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. *J. Dairy Sci.* **2009**, *92*, 5199-211. <https://doi.org/10.3168/jds.2009-2375>
50. Dijkstra, J.; Van Zijderveld, S.M.; Apajalahti, J.A.; Bannink, A.; Gerrits W.J.; Newbold, J.R.; Perdok, H.B.; Berends, H. Relationships between methane production and milk fatty acid profiles in dairy cattle. *Anim. Feed Sci. Technol.* **2011**, *166*, 590-595. <https://doi.org/10.1016/j.anifeedsci.2011.04.042>
51. Rico, D.E.; Chouinard, P.Y.; Hassanat, F.; Benchaar, C.; Gervais, R. Prediction of enteric methane emissions from Holstein dairy cows fed various forage sources. *Animal*, **2016**, *10*, 203-211. <https://doi.org/10.1017/S1751731115001949>
52. Palmquist, D.L.; Beaulieu, A.D.; Barbano, D.M. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* **1993**, *76*, 1753–1771, [https://doi.org/10.3168/jds.S0022-0302\(93\)77508-6](https://doi.org/10.3168/jds.S0022-0302(93)77508-6)
53. Odongo, N.E.; Or-Rashid, M.M.; Kebreab, E.; France, J.; McBride, B.W. Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. *J. Dairy Sci.* **2007**, *90*, 1851-1858. <https://doi.org/10.3168/jds.2006-541>
54. Hristov, A.N.; Vander Pol, M.; Agle, M.; Zaman, S.; Schneider, C.; Ndegwa, P.; Vaddella, V.K.; Johnson, K.; Shingfield, K.J.; Karnati, S.K. Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in lactating cows. *J. Dairy Sci.* **2009**, *92*, 5561-5582. <https://doi.org/10.3168/jds.2009-2383>
55. Oliveira, R.; Faria, M.; Silva, R.; Bezerra, L.; Carvalho, G.; Pinheiro, A.; Simionato, J.; Leão, A. Fatty acid profile of milk and cheese from dairy cows supplemented a

- diet with palm kernel cake. *Molecules*. **2015**, *20*, 15434-15448. <https://doi.org/10.3390/molecules200815434>
56. McNamara, S.; Murphy, J.J.; O'mara, F.P.; Rath, M.; Mee, J.F. Effect of milking frequency in early lactation on energy metabolism, milk production and reproductive performance of dairy cows. *Livest. Sci.* **2008**, *117*: 70–78, [https://doi.org/10.3168/jds.S0022-0302\(06\)72215-9](https://doi.org/10.3168/jds.S0022-0302(06)72215-9)
57. Tiplady, K.M.; Lopdell, T.J.; Sherlock, R.G.; Johnson, T.J.; Spelman, R.J.; Harris, B.L.; Davis, S.R.; Littlejohn, M.D.; Garrick, D.J. Comparison of the genetic characteristics of directly measured and Fourier-transform mid-infrared-predicted bovine milk fatty acids and proteins. *J. Dairy Sci.* **2022**, *105*, 9763-91. <https://doi.org/10.3168/jds.2022-22089>
58. De Marchi, M.; Fagan, C.C.; O'donnell, C.P.; Cecchinato, A.; Dal Zotto, R.; Cassandro, M.; Penasa, M.; Bittante, G. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. *J. Dairy Sci.* **2009**, *92*, 423-32. <https://doi.org/10.3168/jds.2008-1163>
59. De Marchi, M.; Penasa, M.; Tiezzi, F.; Toffanin, V.; Cassandro, M. Prediction of milk coagulation properties by Fourier transform mid-infrared spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability. In Proceedings of the 38th International Committee for Animal Recording (ICAR) Meeting, Cork, Ireland, May 2012.
60. De Marchi, M.; Toffanin, V.; Cassandro, M.; Penasa, M. Prediction of coagulating and noncoagulating milk samples using mid-infrared spectroscopy. *J. Dairy Sci.* **2013**, *96*, 4707-15. <https://doi.org/10.3168/jds.2012-6506>
61. Delamaire, E.; Guinard-Flament, J. Increasing milking intervals decreases the mammary blood flow and mammary uptake of nutrients in dairy cows. *J. Dairy Sci.* **2006**, *89*, 3439–3446, [https://doi.org/10.3168/jds.S0022-0302\(06\)72381-5](https://doi.org/10.3168/jds.S0022-0302(06)72381-5)
62. Morris, C.; Cullen, N.; Glass, B.; Hyndman, D.; Manley, T.; Hickey, S.; McEwan, J.; Pitchford, W.; Bottema, C.; Lee, M. Fatty acid synthase effects on bovine adipose fat and milk fat. *Mamm. Genome*. **2007**, *18*, 64-74. <https://doi.org/10.1007/s00335-006-0102-y>
63. Stoop, W.M.; Schennink, A.; Visker, M.H.; Mullaart, E.; Van Arendonk, J.A.; Bovenhuis, H. Genome-wide scan for bovine milk-fat composition. I. Quantitative trait loci for short-and medium-chain fatty acids. *J. Dairy Sci.* **2009**, *92*, 4664-4675. <https://doi.org/10.3168/jds.2008-1966>

64. Bouwman, A.C.; Visker, M.H.P.W.; van Arendonk, J.M.; Bovenhuis, H. Fine mapping of a quantitative trait locus for bovine milk fat composition on Bos taurus autosome 19. *J. Dairy Sci.* **2014**, *97*, 1139-1149. <https://doi.org/10.3168/jds.2013-7197>
65. Summer, A.; Malacarne, M.; Martuzzi, F.; Mariani, P. Structural and functional properties of Modenese cow milk in Parmigiano-Reggiano cheese production. *Ann. Fac. Med. Vet. Univ. Parma.* **2002**, *22*, 163–174.
66. Martin, B.; Pomies, D.; Pradel, P.; Verdier-Metz, I.; Rémond, B. Yield and sensory properties of cheese made with milk from Holstein or Montbéliarde cows milked twice or once daily. *J. Dairy Sci.* **2009**, *92*, 4730–4737. <https://doi.org/10.3168/jds.2008-1914>.



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:	Inthujaa Sanjayanaraj		
Name and title of main supervisor:	Prof Nicolas Lopez-Villalobos		
In which chapter is the manuscript/published work?	Chapter 3		
What percentage of the manuscript/published work was contributed by the student?	90%		
Describe the contribution that the student has made to the manuscript/published work: The student performed the data analysis, interpreted the results, and wrote the first draft of the paper following the comments and advice of the co-authors.			
Please select one of the following three options:			
<input checked="" type="radio"/>	<b>The manuscript/published work is published or in press</b> Please provide the full reference of the research output: Sanjayanaraj, I.; Lopez-Villalobos, N.; Blair, H.T.; Janssen, P.W.M.; Holroyd, S.E.; MacGibbon, A.K.H. 2022. Fatty acid composition of dairy Milk: A case study comparing once- and twice-a-day milking of pasture-fed cows at different stages of lactation. Dairy, 3, 174–189. <a href="https://doi.org/10.3390/dairy3010014">https://doi.org/10.3390/dairy3010014</a>		
<input type="radio"/>	<b>The manuscript is currently under review for publication</b> Please provide the name of the journal:		
<input type="radio"/>	<b>It is intended that the manuscript will be published, but it has not yet been submitted to a journal</b>		
Student's signature:	Inthujaa Sanjayanaraj <small>Digitally signed by Inthujaa Sanjayanaraj DN: cn=Inthujaa Sanjayanaraj, o=Massey University, New Zealand, ou=School of Agriculture and Environment, email=Sanjayanaraj@massey.ac.nz Date: 2023.04.28 10:34:47 +1200</small>	Main supervisor's signature:	Nicolas Lopez-Villalobos <small>Digitally signed by Nicolas Lopez-Villalobos DN: cn=Nicolas Lopez-Villalobos, o=Massey University, ou=School of Agriculture and Environment, email=N.Lopez-Villalobos@massey.ac.nz Date: 2023.04.28 07:11:37 +1200</small>
<i>This form should be placed at the beginning of each relevant thesis chapter.</i>			





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:	Inthujaa Sanjayananj
Name and title of main supervisor:	Prof Nicolas Lopez-Villalobos
In which chapter is the manuscript/published work?	Chapter 4
What percentage of the manuscript/published work was contributed by the student?	90%
Describe the contribution that the student has made to the manuscript/published work: The student performed the data analysis, interpreted the results, and wrote the first draft of the paper following the comments and advice of the co-authors.	
Please select one of the following three options:	
<input checked="" type="radio"/>	<p><b>The manuscript/published work is published or in press</b></p> <p>Please provide the full reference of the research output: Sanjayananj, I.; Lopez-Villalobos, N.; Blair, H.T.; Janssen, P.W.M.; Holroyd, S.E.; MacGibbon, A.K.H. 2022. Effect of breed on the fatty acid composition of milk from dairy cows milked once and twice a day in different stages of lactation. Dairy, 3, 608–621. <a href="https://doi.org/10.3390/dairy3030043">https://doi.org/10.3390/dairy3030043</a></p>
<input type="radio"/>	<p><b>The manuscript is currently under review for publication</b></p> <p>Please provide the name of the journal:</p>
<input type="radio"/>	<p><b>It is intended that the manuscript will be published, but it has not yet been submitted to a journal</b></p>
Student's signature:	<p>Inthujaa Sanjayananj</p> <p><small>Digitally signed by Inthujaa Sanjayananj DN: cn=Inthujaa Sanjayananj, c=NZ, o=Massey University, New Zealand, ou=School of Agriculture and Environment, email=I.Sanjayananj@massey.ac.nz Date: 2023.04.28 13:06:31 +12'00'</small></p>
Main supervisor's signature:	<p>Nicolas Lopez-Villalobos</p> <p><small>Digitally signed by Nicolas Lopez-Villalobos DN: cn=Nicolas Lopez-Villalobos, c=NZ, o=Massey University, ou=School of Agriculture and Environment, email=N.Lopez-Villalobos@massey.ac.nz Date: 2023.04.28 07:12:29 +12'00'</small></p>
<i>This form should be placed at the beginning of each relevant thesis chapter.</i>	





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:	Inthujaa Sanjayananj		
Name and title of main supervisor:	Prof Nicolas Lopez-Villalobos		
In which chapter is the manuscript/published work?	Chapter 5		
What percentage of the manuscript/published work was contributed by the student?	90%		
Describe the contribution that the student has made to the manuscript/published work: The student performed the data analysis, interpreted the results, and wrote the first draft of the paper following the comments and advice of the co-authors.			
Please select one of the following three options:			
<input checked="" type="radio"/>	<b>The manuscript/published work is published or in press</b> Please provide the full reference of the research output: Sanjayananj, I.; MacGibbon, A.K.H.; Holroyd, S.E.; Janssen, P.W.M.; Blair, H.T.; Lopez-Villalobos, N. 2023. Association of single nucleotide polymorphism in the DGAT1 gene with the fatty acid composition of cows milked once and twice a day. <i>Genes</i> , 14, 767. <a href="https://doi.org/10.3390/genes14030767">https://doi.org/10.3390/genes14030767</a>		
<input type="radio"/>	<b>The manuscript is currently under review for publication</b> Please provide the name of the journal:		
<input type="radio"/>	<b>It is intended that the manuscript will be published, but it has not yet been submitted to a journal</b>		
Student's signature:	Inthujaa Sanjayananj <small>Digitally signed by Inthujaa Sanjayananj DN: cn=Inthujaa Sanjayananj, o=KZ, ou=Massey University, New Zealand, ou=School of Agriculture and Environment, email=I.Sanjayananj@massey.ac.nz Date: 2023.04.20 13:12:00 +1200</small>	Main supervisor's signature:	Nicolas Lopez-Villalobos <small>Digitally signed by Nicolas Lopez-Villalobos DN: cn=Nicolas Lopez-Villalobos, o=KZ, ou=Massey University, ou=School of Agriculture and Environment, email=N.Lopez-Villalobos@massey.ac.nz Date: 2023.04.28 07:12:47 +1200</small>
<i>This form should be placed at the beginning of each relevant thesis chapter.</i>			





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:	Inthujaa Sanjayanraj
Name and title of main supervisor:	Prof Nicolas Lopez-Villalobos
In which chapter is the manuscript/published work?	Chapter 6
What percentage of the manuscript/published work was contributed by the student?	90%
Describe the contribution that the student has made to the manuscript/published work: The student performed the data analysis, interpreted the results, and wrote the first draft of the paper following the comments and advice of the co-authors.	
Please select one of the following three options:	
<input checked="" type="radio"/>	<p><b>The manuscript/published work is published or in press</b></p> <p>Please provide the full reference of the research output: Sanjayanraj, I.; Lopez-Villalobos, N.; Blair, H.T.; Janssen, P.W.M.; Holroyd, S.E.; MacGibbon, A.K.H. 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. <a href="https://doi.org/10.3390/dairy4010012">https://doi.org/10.3390/dairy4010012</a></p>
<input type="radio"/>	<p><b>The manuscript is currently under review for publication</b></p> <p>Please provide the name of the journal:</p>
<input type="radio"/>	<p><b>It is intended that the manuscript will be published, but it has not yet been submitted to a journal</b></p>
Student's signature:	<p><b>Inthujaa Sanjayanraj</b></p> <p><small>Digitally signed by Inthujaa Sanjayanraj DN: cn=Inthujaa Sanjayanraj, o=MZ, ou=Massey University, New Zealand, ou=School of Agriculture and Environment, email=Inthujaa.Sanjayanraj@massey.ac.nz Date: 2023.04.26 13:14:06 +12'00'</small></p>
Main supervisor's signature:	<p><b>Nicolas Lopez-Villalobos</b></p> <p><small>Digitally signed by Nicolas Lopez-Villalobos DN: cn=Nicolas Lopez-Villalobos, o=MZ, ou=Massey University, ou=School of Agriculture and Environment, email=N.Lopez-Villalobos@massey.ac.nz Date: 2023.04.28 07:13:02 +12'00'</small></p>
<i>This form should be placed at the beginning of each relevant thesis chapter.</i>	