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**Effect of genomic inbreeding on lactation yields of milk,
fat and protein and fertility of grazing dairy cows in two
New Zealand farms**

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

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Tyler Porteous

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Abstract

Inbreeding depression is the reduction in phenotypic performance associated with inbred animals. The objective of this study was to calculate the effect of genomic inbreeding on milk production (milk, fat, protein yields and somatic cell score) and fertility traits (start of mating to first service and 21-day submission rate). Genomic inbreeding was calculated through genomic relationship matrices with a panel of 41,417 single nucleotide polymorphisms from 1,708 Holstein-Friesians (F), 412 Jersey (J) and 2,895 Holstein-Friesian \times Jersey (F \times J) cows. Lactation records and mating records were taken from the 2015 to 2020 production seasons of two New Zealand farms. The average inbreeding Jersey cows had higher levels of inbreeding than F and F \times J cows. The effect of inbreeding was most detrimental on milk yield (-57 L/cow per 1% of inbreeding), but also decreased fat and protein yield (-1.7 kg/cow and -1.7 kg/cow per 1% of inbreeding). However, there was no significant effect of inbreeding on somatic cell score and reproductive performance traits. Further analysis with a larger study population and use of other methods to calculate genomic inbreeding coefficients are recommended for future studies.

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List of Abbreviations

BV	Breeding value
BW	Breeding worth
EV	Economic value
F	Holstein-Friesian
F×J	Holstein-Friesian × Jersey crossbred
J	Jersey
LIC	Livestock Improvement Corporation
MF	Milking frequency
OAD	Once-a-day
SCC	Somatic cell count
SCS	Somatic cell score
SMFS	Start of mating to first service
SNP	Single nucleotide polymorphism
SR21	21-day submission rate
TAD	Twice-a-day

Chapter 1

General introduction

The breeding objective of the breeding program in New Zealand dairy cattle is to improve the genetic merit of the cows to convert feed into profit (Harris 2005). The cows and bulls are selected based on a selection index called Breeding Worth (BW) that measures the genetic superiority or inferiority of a dairy animal to transmit farm profitability expressed as net income per 5 tonnes of feed dry matter (Harris 1998). To improve the quality of the herd, animals are mated according to BW. With the best cows being used each season, inevitably there is mating of highly related animals in order to increase overall genetic merit of the national herd. Inbreeding is the combination in homozygosity of alleles with a common ancestor. Although the effects of inbreeding can be beneficial, bringing high production variations together in successive offspring, there is also an increased probability for detrimental allele combinations to arise. Inbreeding depression is the reduction in phenotypic performance associated with inbred animals (McParland et al. 2007). When selecting for high performing cattle, there is a risk of inbreeding depression where animals BW decreases in offspring. The effect of high inbreeding negatively influences productivity and calving performance, resulting in animals that are inferior to their parents, reducing genetic gain.

Long-term level of inbreeding in New Zealand dairy cattle is controlled through specialised breeding strategies using optimal contribution theory (Meuwissen 1987) when elite cows are mated to elite bulls to produce new young bulls. In this method, selection is a long-term strategy that penalises co-ancestry while aligning high merit individuals together, avoiding the crossing of highly related animals and developing a more beneficial pair. At the commercial level, Livestock Improvement Corporation (LIC) manages inbreeding through DataMATE, a remote tool that uses pedigree information to avoid matings between cows and bulls that produce progeny with more than 6.25% of inbreeding.

The level of inbreeding can now be estimated from the genomic relationship matrix constructed using single nucleotide polymorphism (SNP) arrays (VanRaden 2008). Genomic inbreeding coefficients can be more accurate than pedigree-based measures because they capture the variation due to Mendelian sampling and therefore can differ among individuals with the same pedigree (Keller et al. 2011).

The effect of inbreeding on production and reproductive performance of dairy cattle in other countries is well documented in literature (e.g., Cassell et al. 2003; MacParland et al. 2007; Dezetter et al. 2014). However, this has not been reported in New Zealand dairy cattle.

The objective of this study was to calculate the effect of genomic inbreeding on milk production (milk, fat, protein yields and somatic cell score) and fertility traits (start of mating to first service and 21-day submission rate) in dairy cows in two dairy farms.

Chapter 2

Literature review: Effects of inbreeding on productive and reproductive performance of grazing dairy cattle

2.1 New Zealand milk production systems

Positioned as the 8th largest milk producer worldwide, New Zealand's dairy industry has established itself as a significant contributor to the national economy (Zealand 2021). Responsible for 35% of the world trade in dairy products, New Zealand produced 1.947 billion kg of milksolids (fat and protein) in the 2019-2020 season, a 2.7% increase from the previous years (DairyNZ 2021b). Over the past 30 years, national dairy exports have grown from \$2 to \$20 billion, placing dairy as New Zealand's greatest export sector of 2020. Milk exportation alone directly added 10.2 billion dollars into the economy, regardless of Covid-19 implications in 2021 (Pawson and Perkins 2017; Zealand 2021).

New Zealand's milk production is diverse, allowing for the specificity of products to accommodate a variety of countries and community's needs. Over 1,500 different milk-based goods are derived from New Zealand milk, with the top four exports being whole milk powder (37%), cheese (12%), skim milk powder (10%), and butter (9%). These are predominantly distributed to China, Australia, United States of America and Malaysia (Aschakulporn and Zhang 2021; Zealand 2021).

Over time, the breed composition of the national herd has evolved to favour crossbred types due to the increase in hybrid vigour they offer (DairyNZ 2021b). For the 2020-2021 production season, the New Zealand dairy cattle population was comprised of Holstein-Friesian (F)(32.5%), Jersey (J) (8.2%), Holstein-Friesian × Jersey crossbred (F×J) (49.6%) and Ayrshire (0.4%) (DairyNZ 2021a). The popularity of F×J cows is due to crossbreeding increasing calving ease and fertility offered by the Jersey cow, while maintaining high milk production contributed by both breeds as a result of hybrid vigour (Harris 2005).

New Zealand's unique pastoral environment is ideal for dairy farming, offering an advantage in not only milk production, but also animal health and environmental care. Temperate climate and fertile plains allow for consistent annual grass growth, contributing to high levels of pasture to milk conversion (Verkerk 2003). These low intensity conditions also reduce the effect of disease and health complications, moderate climate allows herds to be outdoors all year round. High intensity indoor farming constrains animals, restricting natural cow behaviour. These conditions can lead to increased levels of lameness and greater risk of disease prevalence (Endres and Schwartzkopf-Genswein 2018). Intense farming systems are also detrimental to the environment, caused by increased levels of pollution and indirect carbon emissions

(Gerssen-Gondelach et al. 2017). Overall, the nature and thus farming types of New Zealand dairy, encourage greater milk production, while taking into consideration the longevity of animals and their environment in order to maximise profit (Verkerk 2003).

Pasture-based production systems are influential in New Zealand dairy farming, efficiently converting grass to profit in a way that is best suited to its environment with little reliance on supplements (Garcia and Holmes 1999; Verkerk 2003). Defined by where and what the animals graze, a pasture-based system relies on grass growth and rotational grazing (Conner et al. 2005). Most New Zealand supply farms align calving, a high feed demand period, with the maximum growth period of grass. The farmers synchronise cows to calve in early Spring and consequently the end of lactation occurring in late Summer/Autumn. This is the period with the lowest pasture growth to occur when cattle have low feed requirements, in a non-lactating period when cows are dried off. Positioning these periods together significantly reduces the need for supplements (Garcia and Holmes 1999). With differing demands of modern-day farmers there is also increased popularity of autumn calving, providing consistent dairy production all year round. Autumn calving entails that the lactation period is synchronised with high demand for feed which is not met with pasture only systems (Clark et al. 2007). Although New Zealand farmers experience a harsher season, the climate is moderate and therefore pasture-based farming is prominent. This reduces additional expenses, and with calving alignment, makes New Zealand's pasture-based systems particularly profitable for dairy farming (Garcia and Holmes 1999; Verkerk 2003).

2.2 Breeding program of New Zealand dairy cattle

In order to improve the profitability of the production system, the industry has designed and implemented a breeding program for the genetic improvement of dairy cows. The breeding objective of this breeding program is to breed dairy cows that efficiently convert feed into profit (Harris 2005). The cows and bulls are ranked based on a selection index called BW that measures the genetic superiority or inferiority of a dairy animal to transmit farm profitability expressed as net income per 5 tonnes of feed dry matter (Harris 1998). BW is calculated with the following formula:

$$\begin{aligned} \text{BW} &= (\text{BV}_{\text{milk}} \times \text{EV}_{\text{milk}} \\ &+ (\text{BV}_{\text{fat}} \times \text{EV}_{\text{fat}} \end{aligned}$$

$$\begin{aligned}
&+ (\text{BV}_{\text{protein}} \times \text{EV}_{\text{protein}} \\
&+ (\text{BV}_{\text{liveweight}} \times \text{EV}_{\text{liveweight}} \\
&+ (\text{BV}_{\text{fertility}} \times \text{EV}_{\text{fertility}} \\
&+ (\text{BV}_{\text{somatic cell score}} \times \text{EV}_{\text{somatic cell score}} \\
&+ (\text{BV}_{\text{functional survival}} \times \text{EV}_{\text{functional survival}} \\
&+ (\text{BV}_{\text{body condition score}} \times \text{EV}_{\text{body condition score}} \\
&+ (\text{BV}_{\text{udder overall}} \times \text{EV}_{\text{udder overall}}
\end{aligned}$$

where BV_i is the breeding value of animal for trait i , and EV_i is its corresponding economic value. The economic value expresses the extent to which the breeding goal is improved by one unit of genetic superiority for that trait, i.e., the economic value for each trait is the additional profit per 5.0 t of dry matter (average quality pasture) for a unit change in breeding value for trait i , all other traits in the objective held constant.

Individual animals can be compared across differing breeds, ages and herds because there is a common genetic base for all breeds. The genetic base is made up of a group of well-recorded cows born in 2005. Their profitability is set as the reference point '0' and all animals are reported in relation to this group. The genetic base is updated every five years.

The economic values for each of the traits are calculated using a farm model. This model considers incomes from milk and beef incomes and operation and replacement costs (DairyNZ 2021a).

The selection scheme of the breeding program of the New Zealand dairy cattle is based on four paths of selection (Rendel and Robertson 1950). Increase in genetic gain is achieved through the partitioning of selection on bulls to breed bulls, bulls to breed cows, cows to breed bulls and cows to breed cows. In order to improve the quality of replacements, a New Zealand bull team is constructed, containing only a small number of the highest quality animals which sire 75% of the national herd (DairyNZ 2021b; LIC 2021). Similarly contract milking cows, dams used to produce the next generation of high performing bulls, undergo intense selection in order to produce calves with the highest rate of genetic gain through consideration of both parents. These animals are selected based on genomic selection and progeny testing, resulting in a small

but reliable group of breeding stock. Paired with artificial reproductive technologies, these systems have reduced generation intervals, allowing the younger and more superior bulls to disseminate the entire active cow population, promoting maximum genetic gain (Harris 2005; Clark et al. 2013). While this breeding plan theoretically would produce the most profitable animals, reducing the number of sires or dams can lead to increased rates of inbreeding, evident in offspring performance (González-Recio et al. 2007; Mc Parland et al. 2007). This is because the breeding animals are repeatedly mated to the same sires and dams, resulting in decreased genetic variation throughout the population (Mc Parland et al. 2007). Over time, selection based purely on estimated breeding values results in offspring from different parents increasing significantly in relatedness, reducing fitness.

The detrimental effects of inbreeding on New Zealand dairy cattle are minimised through specialised breeding strategies, allowing genetic variability to be stabilised while promoting substantial genetic gain (Mc Parland et al. 2007; Granleese et al. 2015). Optimal contribution selection is a long-term strategy that penalises co-ancestry while aligning high merit individuals together, avoiding the crossing of highly related animals and developing a more beneficial pair. Combined with reproductive technologies and genomic selection, optimal contribution selection can maintain genetic diversity while maximising genetic gain achieved by intense selection (Clark et al. 2013; Granleese et al. 2015). LIC manages New Zealand dairy cattle inbreeding through DataMATE, a remote tool that uses optimal contribution selection to calculate whether a mating is likely to share recessive alleles or more than 6.25% of their genes. This alerts the artificial insemination technician that the cross is of high risk and an alternative sire will be used (Pitt 2007). With the introduction of imported semen this is particularly useful as the relationship between animals is likely to be unknown to the farmer. Although high levels of selection can increase the effect of inbreeding, this can be managed by specific breeding technology to produce long term genetic gain.

2.3 Inbreeding coefficients

Inbreeding depression is the reduction of an individual's fitness due to fixation of deleterious recessive genes (Mc Parland et al. 2007; Gutiérrez-Reinoso et al. 2020). The crossing of highly related animals results in lengths of homozygosity, evident as reduced genetic variability which increases the risk of mortality (González-Recio et al. 2007; Gutiérrez-Reinoso et al. 2020). The coefficient of inbreeding (F) is the probability that an individual inherits two identical alleles

at the same locus, inferring how inbred an animal is (Bjelland et al. 2013). Inbreeding coefficients can be calculated through both ancestral pedigrees and genomic analysis.

Parentage data can be used to construct large pedigree-based relationship matrices to derive inbreeding coefficients. Analysis of inbreeding in large populations poses difficulty due to the need for a simple and accurate, but high throughput method to calculate F . The numerator relationship matrix (\mathbf{A}) combines additive genetic relationships between individuals, allowing the calculation of inbreeding coefficients and prediction of breeding values using the best linear unbiased predictions (BLUP) method (Henderson 1976; Quaas 1976). A column of the \mathbf{A} matrix represents the genetic relationship of an animal with the other animals in the pedigree. The diagonal elements of the matrix \mathbf{A} represent the degree of inbreeding of each animal in the pedigree. Such calculations of inbreeding predict only the expected proportion of an animal's genome that is considered identical by descent (Nietlisbach et al. 2017). Inaccurate estimations can arise if pedigrees are small or incomplete, missing parentage can underestimate actual levels of inbreeding (Nietlisbach et al. 2017; Gutiérrez-Reinoso et al. 2020). With New Zealand's farming system predominately pasture-based, the risk of incorrect matching of dam to offspring is high and therefore using a pedigree-based calculation is likely to give misleading inbreeding coefficients and breeding values.

2.4 Estimation of genomic inbreeding coefficients

To achieve a more accurate and robust estimation of inbreeding, genetic measures can be used in the calculation of inbreeding coefficient. Genomic based inbreeding coefficients can be calculated through SNP relationship matrices, a method where homozygosity is accurately quantified from marker alleles (Villanueva et al. 2021). Using an individual's DNA offers complete and in-depth ancestry as well as accounting for Mendelian sampling error (McParland et al. 2007; Doekes et al. 2019). With the affordability of DNA sequencing and the evolution of computer memory, a large number of SNPs can be genotyped from whole herds with ease (Gutiérrez-Reinoso et al. 2020). Genomic relationship matrices are more suitable for large scale analyses, therefore would be ideal in assessing the effect of inbreeding on important traits in New Zealand dairy cattle.

VanRaden devised three methods to calculate the genomic relationship matrix (\mathbf{G}) (VanRaden 2008). To show which SNP marker alleles each animal has inherited, an \mathbf{M} matrix is constructed through comparison of the number of individual animals (n) and number of SNP

loci (m). Allele frequencies of each animal are incorporated into the \mathbf{M} matrix with the elements of \mathbf{M} being -1 and 1 if homozygous and 0 if heterozygous for each specific SNP loci. The diagonals of the matrix $\mathbf{M}\mathbf{M}'$ represent the number of homozygous loci for each animal while the off-diagonals show the number of alleles shared between animals. The diagonal elements of the matrix $\mathbf{M}'\mathbf{M}$ count the number of homozygous individuals for each locus and the off-diagonals express the number of instances where alleles at different loci were inherited by the same animal. In the calculation of genomic inbreeding coefficients, a \mathbf{P} matrix is required. This \mathbf{P} matrix expresses the allele frequency of the second allele at each SNP expressed as follows,

$$\mathbf{P} = [2(\mathbf{p}_1 - 0.5) \quad 2(\mathbf{p}_2 - 0.5) \quad \dots \quad 2(\mathbf{p}_m - 0.5)]$$

where \mathbf{p}_i is the column vector of the frequency of the second allele at SNP i .

Then a matrix \mathbf{Z} is calculated as

$$\mathbf{Z} = \mathbf{M} - \mathbf{P}$$

The first method is an extension from the pedigree matrix, with \mathbf{G} being scaled to be analogous to the \mathbf{A} matrix. The \mathbf{G} matrix is calculated using the following formula:

$$\mathbf{G} = \frac{\mathbf{Z}\mathbf{Z}'}{2 \sum p_i(1 - p_i)}$$

Inbreeding coefficient of a particular animal is calculated as $G_{jj}-1$, where G_{jj} is the diagonal elements of the matrix \mathbf{G} . Relatedness between animals is derived through division of elements of G_{jk} by the square roots of G_{jj} and G_{kk} diagonals (Wright 1922; VanRaden 2008).

The second method extends from the original equation, taking into consideration the weighting of marker loci by their expected variance, promoting more rare marker SNPs that will then contribute more to the resulting \mathbf{G} and \mathbf{F} values (VanRaden 2008). Inbreeding coefficient for animal j is again calculated as $G_{jj}-1$. The matrix \mathbf{G} is calculated with the following equation.

$$\mathbf{G} = \mathbf{Z}\mathbf{D}^{-1}\mathbf{Z}'$$

where \mathbf{D} is a diagonal with elements

$$D_{ii} = \frac{1}{m[2p_i(1 - p_i)]}$$

The third method proposed by VanRaden (2008) considers mean homozygosity of individuals through the regression of \mathbf{MM}' which in turn generates \mathbf{G} without the need for allele frequencies, that is

$$\mathbf{G} = \frac{\mathbf{MM}' - g_0(\mathbf{1}\mathbf{1}')}{g_1}$$

where g_0 is the intercept and g_1 is the slope, calculated as

$$g_0 = \sum(p_i - q_i)^2$$

$$g_1 = \sum 2p_iq_i$$

This method is ideal if full DNA sequences cannot be obtained and only a subset of marker SNPs were genotyped.

2.5 Effect of inbreeding

With the focus on genetic improvement of production-based traits, it is important to consider the effects of inbreeding when constructing selection schemes. This allows for optimisation of production, fertility and survival in order to enhance profitability of milk production systems. The effect of inbreeding on production traits has been well documented in F dairy cattle (Table 2.1) depicting that as inbreeding increases milk production and fertility is reduced (Cassell et al. 2003; Mc Parland et al. 2007). Although a similar relationship has been reported in J animals, the effect of inbreeding has a greater influence on milk yield in F cows (Maiwashe et al. 2008; Pryce et al. 2014). Other studies have reported that fat and protein yield follow the same trend as milk yield, but inbreeding has substantially less influence on change of yield, resulting in small regression coefficients (Dezetter et al. 2015; Doekes et al. 2019). Increases in inbreeding coefficients have been associated with larger levels of somatic cell counts (SCC) in milk, with greater incidence seen in older animals (Mc Parland et al. 2007). Contrary to these findings, some studies have found no significant effect on inbreeding on SCC (Rokouei et al. 2010; Dezetter et al. 2015).

Table 2.1 Effect of inbreeding on milk production traits of dairy cows worldwide.

Trait	Effect per 1% inbreeding	Breed ¹	Country ²	Reference
Milk production ³				
Milk, kg	-36	F	USA	Cassell et al. (2003)
	-30	F	IRL	McParland et al. (2007)
	-48	F	NL	Doekes et al. (2019)
	-15	J	SA	Maiwashe et al. (2008)
	-21	F	IR	Rokouei et al. (2010)
	-47	F	USA	Bjelland et al. (2013)
	-41	F	FR	Dezetter et al. (2014)
Fat, kg	-1.55	F	NL	Doekes et al. (2019)
	-0.64	J	SA	Maiwashe et al. (2008)
	-0.50	F	IR	Rokouei et al. (2010)
	-2.40	F, J	AU	Pryce et al. (2014)
	-1.70	F	FR	Dezetter et al. (2014)
Protein, kg	-1.06	F	NL	Doekes et al. (2019)
	-0.59	J	SA	Maiwashe et al. (2008)
	-0.52	F	IR	Rokouei et al. (2010)
	-3.45	F, J	AU	Pryce et al. (2014)
	-1.30	F	FR	Dezetter et al. (2014)
Somatic cell score	0.860 ⁴	F	NL	Doekes et al. (2019)
	0.005 ⁵	F	IR	Rokouei et al. (2010)
	0.001 ⁵	F	USA	Bjelland et al. (2013)
	0.006 ⁵	F	FR	Dezetter et al. (2014)

¹ F = Holstein-Friesian, J = Jersey.² USA = United States of America, IRL = Ireland, NL = Netherlands, SA = South Africa, IR = Iran, FR = France, AU = Australia.³ Milk production traits are measured in kg/lactation.⁴ Somatic cell score defined as $SCS = [\log_2(SCC/100,000)]$.⁵ Somatic cell count defined as $SCS = 1000 + 100[\log_2 \text{ of cells/mL}]$.

Inbreeding has a negative effect on fertility traits, including calving to conception interval, conception rate, calving ease, calving interval, age at first calving, interval from first to last insemination and days to service (Table 2.2). The time taken between calvings is an important indication of herd fertility as it depicts how quickly and easily a cow can be put back into calf. Late calvings reduce the time allowed for animals to recover before mating. These animals spend more time in late lactation which yields significantly less milk solids than early, reducing overall fertility and production (Doekes et al. 2019; Temesgen et al. 2022). Calving to conception interval and calving interval are both highly indicative of fertility success, with an increase associated with poor ability to return in calf (Temesgen et al. 2022). Both Jersey and Holstein-Friesian breeds expressed similar increase in these traits, ranging between 0.29 - 1.06 days (Rokouei et al. 2010; Bjelland et al. 2013). Conception rate shows the percentage of artificial insemination success in a 21-day mating period (Gutiérrez-Reinoso et al. 2020). This trait is significantly reduced with a 1% increase in inbreeding, with the largest difference being -0.53% in Holstein cattle (Bjelland et al. 2013; Doekes et al. 2019). Other traits such as age at first calving, interval from first to last insemination and days to service show significant deleterious effects from inbreeding but with much smaller changes observed in fertility values. The discovery that inbreeding has a profound effect on an animal's fertility is well supported and likely to have a similar influence in New Zealand dairy cattle.

Health and survivability of dairy cattle is significantly influenced by the level of inbreeding, explored through traits such as survival rate, days of productive life, days in milk and herd life (Table 2.3). The effect of inbreeding depression is generally associated with reduced fitness, in the survival of dairy cattle this is explained through milk production, thus time spent in lactation is influential. Cows that are dry or no longer easily put in calf are often culled, therefore survivability is measured until the end of an animal's productive lifespan (Mc Parland et al. 2007).

Table 2.2 Effect of inbreeding on fertility traits of dairy cows worldwide.

Trait ¹	Effect per 1% inbreeding	Breed ²	Country ³	Reference
Conception rate, %	-0.53	HF	USA	Bjelland et al. (2013)
	-0.36	F	NL	Doekes et al. (2019)
	-0.27	F	FR	Dezetter et al. (2014)
Calving interval, days	0.70	F	IRL	McParland et al. (2007)
	0.62	F	NL	Doekes et al. (2019)
	0.29	F	IR	Rokouei et al. (2010)
	0.62	F	AU	Pryce et al. (2014)
Days to service, days	-0.15	F	USA	Cassell et al. (2003)
AFC, days	0.12	F	FR	Dezetter et al. (2014)
	0.20	F	IRL	McParland et al. (2007)
	0.13	F	IR	Rokouei et al. (2010)
CCI, days	1.06	F	USA	Bjelland et al. (2013)
IFL, days	0.40	F	NL	Doekes et al. (2019)

¹ AFC = age at first calving, CCI = conception to calving interval and IFL= interval from first to last insemination.

² F = Holstein-Friesian.

³ USA = United States of America, NL = Netherlands, FR = France, IRL = Ireland, IR = Iran, AU = Australia.

Survival rate is defined as the proportion of animals that survived to second lactation, depicting number of cull cows. With a significant reduction in survival rate of -0.3% per 1% of inbreeding, more animals would be culled due to poor reproductive ability (Mc Parland et al. 2007). This is correlated to decreases in days of productive life (-6 days) and days in milk (-4.8 days) per 1% inbreeding change, both similar traits indicative of the actual lifespan of a dairy cow (Smith et al. 1998). Herd life is a trait used to represent survivability of an individual, considering the average lifespan of cattle correcting for herd specific conditions. In response to 1% increase in inbreeding coefficient, herd life decreased by 11 days, significantly influencing potential milk production and lifetime of individuals (Bonczek and Young 1980).

Although significant relationships between survivability traits and inbreeding have been found, evidence for such effects is limited and therefore is of interest to further explore. While these studies explored inbred dairy cattle in multiple European and American regions with differing pasture systems, research is yet to investigate the effects of inbreeding on New Zealand's unique dairy cattle milk production, fertility and survival.

Table 2.3 Effect of inbreeding on survivability traits of dairy cows worldwide.

Trait ¹	Effect per 1% inbreeding	Breed ²	Country ³	Reference
Survivability				
Survival rate, %	-0.3	F	IRL	McParland et al. (2007)
Days in milk, days	-4.8	F	USA	Smith et al. (1998)
Herd life, days	-11.0	F	USA	Bonczek et al. (1980)
DPL, days	-6.0	F	USA	Smith et al. (1998)

¹ DPL = Days of productive life.

² F = Holstein-Friesian.

³ IRL = Ireland, USA = United States of America.

2.6 Research objective

With the influence of the New Zealand dairy industry on national economy, it is important that milk production and fertility is optimised by genetic improvement. The effect of genomic inbreeding on milk production (milk, fat, protein yields and somatic cell score) and fertility traits (start of mating to first service and 21-day submission rate) is unknown in New Zealand dairy cattle. Therefore, the objective of this study is to determine the extent of inbreeding within two dairy herds and determine whether increased inbreeding coefficient is associated with reductions in performance.

Chapter 3

Material and methods

3.1 Farm and herd structure

The data was collected from Massey University's farms Dairy 1 and Dairy 4, situated in Palmerston North. Dairy 1 (-40.380620 latitude, 175.615220 longitude) is a low-input farm with once-a-day (OAD) milking and spring calving. A pasture-based production system is adopted with farmland containing ryegrass in addition to white and red clover mix (100 hectares), plantain and chicory with white and red clover mix (10 hectares) and lucerne (10 hectares). Dairy 4 (-40.378500 latitude, 175.609910 longitude) is a high-input farm with twice-a-day (TAD) milking and spring calving. This pasture-based farm is mostly perennial ryegrass and white clover. Cows in Dairy 1 were milked at 6:30 am while cows in Dairy 4 cows were milked at 5:30 am and 2:30 pm. Both farms' breeding period occurred between October 18th and finished on December 23rd, with calving commencing in mid-July and milking continuing until May of the following year.

A total of 4,167 lactation records were used in this study. Animals consisted of F, J and F×J cows calving between 2015 and 2020. Cows with proportion of F or J \geq 87.5% were considered as F and J, respectively, the remainder of the cows were considered as F×J crossbreds. Dairy 1 cows included approximately 25% F, 25% J and 50% F×J breeds. In comparison, Dairy 4 cows were predominately F and F×J crossbreds. Number of cows per farm, production season and breed are presented in Table 3.1.

Table 3.1. Number of cows in the study population for each farm, season and breed¹.

Season	Dairy 1				Dairy 4			
	F	F×J	J	Total	F	F×J	J	Total
2015	70	127	65	262	151	430	12	593
2016	69	137	56	262	182	402	7	591
2017	60	128	58	246	209	370	4	583
2018	54	124	64	242	242	322	4	568
2019	62	126	69	257	256	320	5	581
2020	75	122	62	259	278	287	6	571

¹ F = Holstein-Friesian, J = Jersey and F×J = Holstein-Friesian×Jersey crossbred.

3.2 Parentage data collection

A pedigree file was retrieved from Minda online database, where ancestral records were kept from each breeding season. Calves were parentage-verified through DNA each season.

Ear punch tissue samples were taken in order to extract DNA which was genotyped using Bovine Illumina 50K SNP-chips. Quality control of SNPs and animals was carried out using the SNP & Variation Suite as described by Ariyaratne (Ariyaratne et al. 2021). The genotypes recorded in Illumina A/B allele format were converted to -1, 0 or 1, depending on the number of B alleles present at each locus. Loci with a call rate $\leq 80\%$ or minor allele frequency ≤ 0.01 , as well as animals with a call rate $\leq 80\%$. After these QC steps, a total of 41,417 SNPs qualified for the calculation of genomic inbreeding coefficients.

3.3 Milk production traits

The 4,167 lactation records from cows in Dairy 1 and Dairy 4 were collected from 2015 to 2020. Traits analysed were lactation yields of milk, fat and protein and average SCS. The average SCS for each lactation was the average of SCS calculated for each herd during the lactation, calculated as $SCS = \text{Log}_2(\text{somatic cell count})$.

3.4 Fertility traits

Both herds had a season calving with mating period during October to December of each year. Tail painting was used for heat detection. Each service date was recorded. Pregnancy diagnosis was performed at the end of the breeding season using ultrasound scanning equipment. Fertility traits considered in this study were interval (days) from date of start of mating of the herd to first service (SMFS) and presentation or non-presentation for mating within the first 21 days of the start of the mating (SR21).

3.5 Inbreeding coefficients

Inbreeding coefficients were estimated using the PROC IML programming language of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) in accordance with VanRaden (2008) third method to estimate the genomic relationship matrix **G** with the SNP genotypes, that is,

$$\mathbf{G} = \frac{\mathbf{MM}' - g_0(\mathbf{1}\mathbf{1}')}{g_1}$$

where \mathbf{M} is a matrix of 1577 rows (number of animals) and 41,417 columns (number of SNPs) with elements -1, 0 and 1 coding SNP genotypes (-1 and 1 for homozygous and 0 for heterozygous for each specific SNP loci), and g_0 and g_1 calculated as

where g_0 is the intercept and g_1 is the slope, calculated as

$$g_0 = \sum(p_i - q_i)^2$$

$$g_1 = \sum 2p_iq_i$$

3.6 Statistical analysis

All statistical analysis were performed using the SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). Cows were classified into four classes of level of inbreeding (Table 3.2).

Effects of genomic inbreeding on production and fertility traits were evaluated with two mixed models. The first model included the fixed effects of farm (Dairy 1 and Dairy 4), production season (2015 to 2020), lactation number (1, 2 and 3+), and breed within farm, deviation from median calving date in the farm and production season and coefficient of genomic inbreeding as covariates, and the random effects of cow and residual errors assumed with zero means and variances σ_c^2 and σ_e^2 , respectively. The second model was the same as the first model, but inbreeding effects were considered as class effects (zero, low, mid and high).

Least-squares means for each level of the fixed effect were obtained and used for multiple mean comparisons using Fisher's least significant difference. Significant differences between means were declared at $P < 0.05$.

Table 3.2: Number of cows classified according to level of inbreeding.

Class of inbreeding											
Zero (inbreeding=0%)			Low (0<inbreeding≤4%)			Mid (4<inbreeding≤8%)			High (inbreeding>8%)		
F	F×J	J	F	F×J	J	F	F×J	J	F	F×J	J
292	499	1	184	145	4	44	63	15	5	21	91

Chapter 4
Results

4.1 Descriptive statistics

Descriptive statistics for the production and fertility traits are presented in Table 4.1. The coefficients of variation for the milk production traits and SCS were similar to each other (22% to 25%), but the coefficients of variation for fertility traits were greater (32% and 69%).

Level of inbreeding in the cow population, calculated through the genomic relationship matrix method is displayed in Figure 4.1. The cow population is significantly skewed to the right, in favour of low levels of inbreeding with 67% of animals expressing an inbreeding value between 0-8% with more extreme values extending to 22% inbred.

The relationship between inbreeding coefficient and milk production for each breed group is presented in Figure 4.2. Holstein-Friesian cows express a slightly negative relationship between level of inbreeding and milk production. These animals are relatively dispersed, with most values clustered to the left, favouring low levels of inbreeding with moderate milk production. Some extreme points are present, showing high levels of inbreeding with reduced milk production. A similar relationship is presented within the J breed, as inbreeding increased, milk production moderately decreased. Majority of values are clustered in the lower centre of the graph, meaning Jerseys on average had higher levels of inbreeding but the decrease in milk production was similar across other breeds. The regression coefficient of milk production on coefficient of inbreeding in $F \times J$ crossbred cows is more negative than in F and J cows, although not expressing high levels of inbreeding, the change in milk yield per 1% is considerably more than the other breeds. Similar to F, the crossbred animals are clustered to the left, with the average level of inbreeding in animals being low.

Table 4.1. Descriptive statistics¹ for milk production and fertility traits of cows at Dairy 1 and Dairy 4 during the 2015 to 2020 production seasons at Massey University.

Trait	Mean	SD	CV %	Minimum	Maximum
Milk production,					
kg/lactation					
Milk	5,084	1,261	25	562	8,861
Fat	236	51	22	37	398
Protein	193	42	22	25	311
Milksolids (fat plus protein)	429	90	21	62	695
Somatic cell score ²	5.400	1.200	22	2.7	11.0
Somatic cell count, [(10 ³ /ml)] ³	40			6.5	2048
Fertility ⁴					
SMFS, days	10.9	7.5	69	0	69
SR21, %	90	29	32		
Inbreeding, %	1.9	3.9	-	0	22.2

¹ SD = standard deviation, CV = coefficient of variation.

² Somatic cell score defined as $SCS = 1000 + 100[\log_2 \text{ of cells/mL}]$

³ Somatic cell count back transformed as $SCC = 2^{SCS}$

⁴ SMFS = start of mating to first service, SR21 = presentation or non-presentation for mating within the first 21 days of the start of the mating.

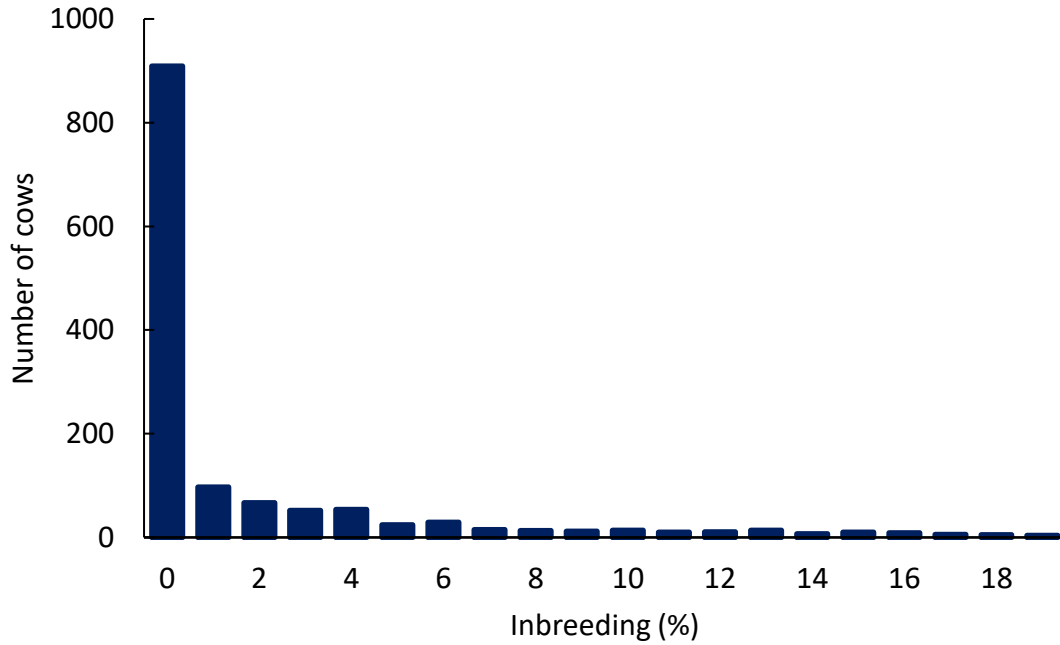


Figure 4.1. Distribution of inbreeding level in dairy cattle from Massey University farms Dairy 1 and 4 from production seasons 2015 to 2020 categorized by breed.

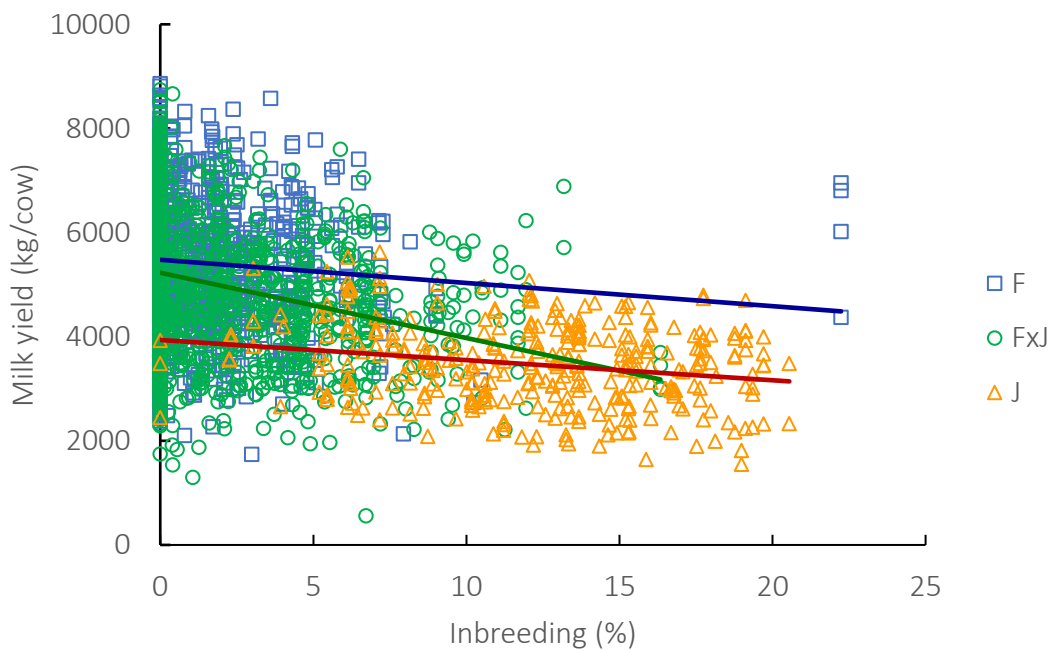


Figure 4.2. Regression of lactation milk yield on level of inbreeding in pasture-based Holstein-Friesian (F), Jersey (J) and crossbred F×J cows at Massey University dairy farms in the production seasons 2015 to 2020.

4.2 Breed performance

Table 4.2 presents the least squares means for production and fertility traits of the cattle population for each breed within the two farms. Findings suggest Dairy 4 (TAD) has greater production than Dairy 1 (OAD) in milk (4601 v 3709 L/cow), fat (220 v 195 kg/cow), protein (178 v 152 kg/cow) and milksolids yields (399 v 348 kg/cow) due to the differing farm effect. F animals produced significantly greater volume of milk than J and F×J cows in both Dairy 4 (3,881 v 3,544 and 3,701 L/cow) and Dairy 1 (5,051 v 4,008 and 4,745 L/cow) farms (p-value<0.05). Similarly, F animals produced more protein content than J and F×J but these differences were only significant between breeds for Dairy 4 cows (186 v 166 and 181 kg/cow) (p-value>0.05). There was no significant difference between the mean of fat and milksolids yield of each breed (p value>0.05). Somatic cell score also significantly differed between farms, with Dairy 4 cows having smaller values than Dairy 1 cows (5.29 v 5.8 $10^3/\text{ml}^3$). There was no significant difference between breeds for either farm (p-value>0.05). Fertility traits showed opposing relationships between values and farm, with SMFS being smaller in Dairy 4 cows than Dairy 1 cows (9.3 v 13.7 days) while SR21 was greater for Dairy 4 (95 v 84%). Differences between breeds for both traits were not significant.

Table 4.2. Least squares means and standard errors (within brackets) for milk production and fertility of dairy cows from different cow breeds in Massey University Dairy 1 and Dairy 4 farms during the 2015 to 2020 production seasons.

Trait	Dairy 1			Dairy 4		
	F	F×J	J	F	F×J	J
Milk production						
Milk, L/lactation	3,881 ^d (72)	3,701 ^e (57)	3,544 ^f (88)	5,051 ^a (44)	4,745 ^b (40)	4,008 ^c (207)
Fat, kg/lactation	189 ^e (3.3)	196 ^e (2.6)	199 ^{de} (4.1)	220 ^{bc} (2.0)	225 ^{ac} (1.8)	216 ^{cd} (9.5)
Protein, kg/lactation	155 ^{cd} (2.5)	154 ^{cd} (2.0)	149 ^d (3.1)	186 ^a (1.5)	181 ^b (1.4)	166 ^c (7.3)
Somatic cell score, (10 ³ /ml) ³	5.90 ^a (0.10)	5.80 ^a (0.08)	5.60 ^a (0.12)	5.13 ^b (0.06)	5.22 ^b (0.06)	5.52 ^{ab} (0.29)
Milksolids ¹ , kg/lactation	345 ^c (6)	350 ^{bc} (4)	349 ^c (7)	406 ^a (3)	407 ^a (3)	383 ^{ab} (16)
Fertility ²						
SMFS, day	13.7 ^a (0.5)	13.5 ^a (0.4)	14.0 ^a (0.6)	9.5 ^b (0.3)	9.4 ^b (0.3)	9.0 ^b (1.4)
SR21, %	84 ^c (1.9)	85 ^{bc} (1.5)	83 ^c (2.3)	94 ^a (1.2)	95 ^a (1.1)	96 ^{ab} (5.6)

¹ Milksolids = fat plus protein.

² SMFS = start of mating to first service, SR21 = presentation or non-presentation for mating within the first 21 days of the start of the mating.

^{a-f} Means with different superscripts within the row are significantly different (P<0.05).

4.3 Effect of inbreeding

Table 4.3 presents the estimates of the regression coefficients of production and fertility traits on level of inbreeding. Inbreeding had a negative effect on all production traits ($P < 0.05$). A 1% increase of inbreeding had a greater effect on milk yield (-58 L/cow) than fat, protein and milksolid yields (-1.7, -1.7 and -3.4 kg/cow). Fertility traits and SCS were not affected by inbreeding level within the study population.

The effect of inbreeding evaluated as a class variable was similar to the regression analysis (Table 4.3) and findings are presented in Table 4.4. All production traits showed a significant decrease in yield between non inbred (zero level) and low inbred (0-4% F) animals. Inbreeding had the greatest effect on milk yield, decreasing the value by -764 L/cow from zero to high inbred animals (>8% F). A significant negative effect was also found to decrease fat (-23 kg/cow), protein (-23 kg/cow) and milksolids yields (-47 kg/cow). The effect of inbreeding differed between fat and protein yields across inbreeding classes. The negative effect of inbreeding on fat content was nonlinear, present in low level inbred (-10 kg/cow) and highly inbred animals (-14 kg/cow) yet insignificant with mid classed cows. In contrast, protein yield decreased more significantly in only highly inbred animals, with the greatest change in trait occurring between mid-high classed animals (-12 kg/cow). Inbreeding had no effect on SCS, SMFS or SR2, with the values of different classed animals not significantly differing from one another.

Table 4.3. Effect of inbreeding on milk production and fertility traits of cows in Dairy 1 and Dairy 4 during the 2015 to 2020 production seasons at Massey University.

Trait	Effect per 1% inbreeding (SE)	P-value
Milk production, kg/lactation		
Milk	-58 (7.21)	<0.001
Fat	-1.7 (0.31)	<0.001
Protein	-1.7 (0.25)	<0.001
Milksolids (fat plus protein)	-3.4 (0.52)	<0.001
Somatic cell score	-0.003 (0.01)	0.8019
Fertility ¹		
SMFS, day	-0.02 (0.05)	0.6127
SR21, %	0.01 (0.002)	0.9794

¹ SMFS = start of mating to first service, SR21 = presentation or non-presentation for mating within the first 21 days of the start of the mating.

Table 4.4. Least square means of milk production and fertility performance of cows with standard errors shown in brackets, classified by level of inbreeding at Dairy 1 and Dairy 4 during the 2015 to 2020 production seasons at Massey University.

Trait	Level of inbreeding			
	Zero	Low	Mid	High
Milk production				
Milk, L/cow	4,572 ^a (42)	4,423 ^b (50)	4,181 ^c (63)	3,808 ^d (77)
Fat, kg/cow	220 ^a (1.95)	210 ^b (2.30)	211 ^{ab} (2.90)	197 ^b (3.56)
Protein, kg/cow	177 ^a (1.48)	171 ^b (1.75)	166 ^{bc} (2.19)	154 ^c (2.70)
Milksolids kg/cow	397 ^a (3.3)	381 ^b (3.8)	377 ^b (4.8)	350 ^c (5.9)
Somatic cell score	5.459 (0.06)	5.515 (0.07)	5.348 (0.09)	5.410 (0.11)
Fertility ¹				
SMFS, days	11.6 (0.29)	11.9 (0.34)	11.7 (0.41)	11.2 (0.52)
SR21, %	89 (0.01)	89 (0.02)	87 (0.02)	90 (0.02)

¹ SMFS = start of mating to first service, SR21 = presentation or non-presentation for mating within the first 21 days of the start of the mating.

^{a, b, c, d} Means with different superscripts within the row are significantly different ($P < 0.05$).

Chapter 5
Discussion

This study investigated the effect of genomic inbreeding on production and fertility traits in grazing dairy cows from two farms. The level of milk production found in this study (5,084 L/cow) is significantly greater than the average of the national herd (4,287 L/cow) (DairyNZ 2020). A similar trend was observed for fat (236 vs 215 kg/cow), protein (193 vs 170 kg/cow) and milksolids (429 vs 385 kg/cow) yield compared with the New Zealand national average. Somatic cell count was considerably different between the study and national statistics, with the study value ($40 \times 10^3/\text{ml}$) being smaller than the national herd average ($173 \times 10^3/\text{ml}$) insinuating better quality milk (Table 4.1). Differences in these production traits between studies can be explained by varying supplementation intensity and farm conditions.

Overall, the amount of inbreeding present within the New Zealand herds is substantially less than many international countries, shown by differing average inbreeding coefficients of studies (1.93% v 3.05%, 3.6% and 5%) (Mc Parland et al. 2007; Cassell et al. 2003 Dezetter et al. 2015), This is expected, as discussed earlier the New Zealand dairy industry has mechanisms such as LIC's DataMate tool to minimise the crossing of highly related animals within individual's pedigrees. New Zealand farmers also use high performing international sires, reducing the number of crossings from animals within the immediate gene pool, increasing genetic diversity. The level of inbreeding within the farms considered in this study is small and inbreeding coefficients have remained constant over time from 2015-2020 (2.03% v 2.00%).

The effect of inbreeding on milk production is consistent with literature (Table 2.1, Table 4.4). This study found that New Zealand dairy cattle decrease 58 L milk/cow per 1% increase in inbreeding, which is similar to the value reported in the Netherlands (-48 L/cow; Doekes et al. 2019) the USA (-47 L/cow; Bjelland et al. 2013). Effects of inbreeding on fat (-1.7 kg/1% inbreeding) and protein (-1.7 kg/1% inbreeding) productions in this study were similar to the effects reported in France (Dezetter et al. 2015) and the Netherlands (Doekes et al. 2019), but lower than the effects reported in Australian dairy cattle (Pryce et al. 2014). Differences in breeds within each study population is likely the cause for the variation in effects of inbreeding on fat and protein yields. Jersey cattle are more popular in New Zealand and Australia in comparison to other international countries, this breed exhibits more inbreeding than F and F×J, therefore the effect of inbreeding is more profound in these studies as shown by a greater decrease in fat and protein (Figure 4.2). Jersey animals also produce more fat than other breeds, the effect of inbreeding is likely to be more profound for this trait and milksolids, therefore the differences are noticeable in milk and protein yields (Lembeye et al. 2016) (Table 4.4). Jersey

animals are more inbred than other breeds due to significant reduction in population size from 1980. This lack of popularity resulted in less importation of quality semen internationally, therefore bull families were selected from a less diverse population.

The effect of inbreeding as a class variable on milk production traits offered findings similar to other papers (Mc Parland et al. 2007; Dezetter et al. 2015). Milk yield significantly differed between classes, with a -764 L/cow decrease between 'zero' and 'high' inbreeding (4,572 v 3,808 L/cow) (Table 4.5). Similarly, fat, protein and milksolids showed significant variation between the low and high classes with differences of -23 kg/cow (220 v 197 kg/cow), -23 kg/cow (177 v 154 kg/cow) and -47 kg/cow (397 v 350 kg/cow), respectively. These changes are a result of inbreeding, the fixation of deleterious alleles in production traits decrease the amount of lactation yields gained from each cow (Cassell et al. 2003).

The effect of inbreeding depression on fertility-based traits was explored through comparison of start of mating to first service (SMFS), where the study population had a significantly smaller interval, a difference of 7.8 days, than the reported New Zealand average (18.7 days) (Grosshans et al. 1997) (Table 4.1). The submission rate from start of mating to 21 days (SR21) is 10% larger in the study population in comparison to the national herd average (80%), indicating better calving performance within the sample (Craig et al. 2018). CV% of 21-day submission rate is significantly smaller than that of SMFS, therefore there is more reliability in the estimates for 21-day submission rate than SMFS. Differences in SMFS and SR21 between study animals and the national herd are likely due to the farm effect, where differing calving management and individual genetics could have an influence on the values.

Of the investigated traits, SCS, SMFS and SR21 did not show a significant difference in value due to the inbreeding effect ($P > 0.05$) (Table 4.4 and 4.5). Contrary to these findings, studies in other countries have observed significant differences (Bjelland et al. 2013; Doekes et al. 2019). This lack of effect is likely due to fertility-based selection in the national herd, this being the culling of empty or high SCC animals that would exhibit low BW. Through optimal contribution of selection and reproductive technologies, many animals within the herds will have better perceived fertility regardless of inbreeding coefficient. Calving rate will be increased with no interaction from genomics but through practices.

This study considered production records from cows milked OAD (Dairy 1) and TAD (Dairy 4) from different breeds with different levels of supplementary feed (Table 4.3). Mean for milk

production between the farms were significantly different ($P < 0.05$). Effect of milking frequency has been reported by several studies to have significant influence on milk production (Clark et al. 2006; Lembeye et al. 2016). This is shown by the differences in least square means of the herds, where TAD is consistently greater in milk yield (10,307 v 11,957 kg/cow) and milksolid yield (929 v 1048 kg/cow) (Clark et al. 2006). Similar averages were expressed in this study, in particular milk yield (3709 v 4601 kg/cow) and milksolid yield (348 v 399 kg/cow). Variation in production traits was likely due to differences in stocking rate and supplementation. OAD cows are denser per hectare of land thus having less available dry matter to consume and convert into milk (Lembeye et al. 2016). Differences seen in production values within this study are due to changes in supplementation between Dairy 1 and 4. TAD milked animals from Dairy 4 are offered substantially more additional feed, therefore allowing significantly more conversion of feed to product. With such findings it is likely the effect of milking frequency is not solely due to the number of times an animal is milked daily, therefore with the selection of better adapted animals the option of OAD milking is becoming more viable to farmers (Woolford et al. 1982).

Studies reported variation between traits with differing milking frequency (MF) between breeds (Clark et al. 2006; Lembeye et al. 2016). Significant differences were seen between F, J and F×J crossbred animals' milk and milksolid yield, with breed effect having more positive influence on TAD milked herds. TAD animals milk yield between F×J and J was 369% more than the difference present in OAD herds (737 v 157 kg/cow) (Table 4.3). Similarly, the milk yield difference between Dairy 4 and 1 in F and F×J was increased by 70% (306 v 180 kg/cow). These production differences are supported by studies, suggesting that Friesian bred cows are superior to F×J and consequently J (Bryant et al. 2007; Penasa et al. 2010; Coffey et al. 2016; Lembeye et al. 2016). Friesian cattle are the most affected breed by changes in MF, while Jersey cows have less production loss when reduced to OAD. Jersey animals are more versatile and adapted to OAD milking, therefore financial loss is minimised when changing MF (Carruthers et al. 1993; Clark et al. 2006).

Milk composition has been found to differ according to breed and MF, resulting in changes of depicted milk production superiority. When comparing the effect of MF, collectively both fat and protein increased from OAD and TAD, with F fat rising by 13% (220 v 195 kg/cow) (Table 4.3). The OAD population found J cattle to produce more fat than other breeds, in agreement with previous studies (Sneddon et al. 2015; Lembeye et al. 2016). This is an advantage to New

Zealand farmers as producers' payment is based upon fat and protein yield (milksolids), therefore although J animals produce less milk yield (L), they are important in contributing to the overall milk solid total (Lembeye et al. 2016). This supports the superiority of F×J animals in milksolids yield, where the crossbreeding of F and J results in an intermediate that produces greater fat content while retaining milk quantity (Dezetter et al. 2015). Although this relationship is likely, this investigation found differences between breeds not to be significant in TAD animals. This is likely due to the underrepresentation of J animals within Dairy 4, with only 1% of J being present in the population, not due to a change in MF.

There are several methods to calculate genomic inbreeding that were not used in this study (VanRaden 2008; Cortes-Hernández et al. 2021). Analysis of runs of homozygosity is an alternative way to calculate inbreeding coefficients in which contiguous homozygote lengths of DNA can be traced back through generations, resulting in current level of inbreeding of an individual as well as the timing of inbreeding events. Understanding when inbreeding events have occurred can inform how severe and influential this homozygosity is on offspring (Martikainen et al. 2020). Calculating the genotype ratio of observed to expected homozygotic SNPs is another method of accurately obtaining a more robust inbreeding coefficients in smaller populations. Incorporating this value offers further information on ancestry, identifying potential outbreeding between subpopulations and different breeds (Rebelato and Caetano 2018). Both methods give inbreeding coefficients greater value, showing that the amount of inbreeding present in an individual does not infer how great of an effect it has.

Conclusions

The study found that animals from both herds had been inbred, although not as largely as reported in other countries. The results show that inbreeding had the greatest effect on milk volume and less of an influence on milksolids, and no effect on somatic cell count, start of mating to first service and submission rate within the first 21 days of the start of the mating.

Jersey cattle are more inbred than Friesian and F×J due to limited effective population size and lack of international sires within their bull team. In further analysis, a larger study population would offer a more robust investigation into whether an inbreeding effect is seen. Use of other methods to calculate inbreeding coefficients would give a more accurate measure of inbreeding present, therefore would be recommended in future studies.

References

- Ariyaratne HBPC, Correa-Luna M, Blair HT, Garrick DJ, Lopez-Villalobos N. 2021. Identification of genomic regions associated with concentrations of milk fat, protein, urea and efficiency of crude protein utilization in grazing dairy cows. *Genes* 12: 456.
- Aschakulporn P, Zhang JE. 2021. New Zealand whole milk powder options. *Accounting & Finance* 61: 2201-2246.
- Bjelland D, Weigel K, Vukasinovic N, Nkrumah J. 2013. Evaluation of inbreeding depression in Holstein cattle using whole-genome SNP markers and alternative measures of genomic inbreeding. *Journal of Dairy Science* 96: 4697-4706.
- Bonczek R, Young C. 1982. Comparison of production and reproduction traits of two inbred lines of Holstein cattle with attention to the effect of inbreeding. *Journal of Dairy Science* 63: 106-107.
- Bryant JR, López-Villalobos N, Holmes CW, Garrick DJ, Pryce JE, Johnsons DL. 2007. Short Communication: Effect of environment on the expression of breed and heterosis effects for production traits. *Journal of Dairy Science* 90: 1548-1553.
- Carruthers VR, Davis SR, Bryant AM, Copeman PJ. 1993. Response of Jersey and Friesian cows to once a day milking and prediction of response based on udder characteristics and milk composition. *Journal of Dairy Research* 60: 1-11.
- Cassell B, Adamec V, Pearson R. 2003. Effect of incomplete pedigrees on estimates of inbreeding and inbreeding depression for days to first service and summit milk yield in Holsteins and Jerseys. *Journal of Dairy Science* 86: 2967-2976.
- Clark D, Phyn C, Tong M, Collis S, Dalley D. 2006. A systems comparison of once-versus twice-daily milking of pastured dairy cows. *Journal of Dairy Science* 89: 1854-1862.
- Clark D, Caradus J, Monaghan R, Sharp P, Thorrold B. 2007. Issues and options for future dairy farming in New Zealand. *New Zealand Journal of Agricultural Research* 50: 203-221.

- Clark SA, Kinghorn BP, Hickey JM, van der Werf JH. 2013. The effect of genomic information on optimal contribution selection in livestock breeding programs. *Genetics selection evolution* 45: 1-8.
- Coffey EL, Horan B, Evans RD, Berry DP. 2016. Milk production and fertility performance of Holstein, Friesian, and Jersey purebred cows and their respective crosses in seasonal-calving commercial farms. *Journal of Dairy Science* 99: 5681-5689.
- Conner DS, Hamm MW, Smalley SB, Williams D. 2005. Pasture-based agriculture: Opportunities for public research institutions In.
- Cortes-Hernández J, García-Ruiz A, Vásquez-Peláez CG, Ruiz-Lopez FdJ. 2021. Correlation of genomic and pedigree inbreeding coefficients in small cattle populations. *Animals* 11: 3234.
- Craig HJB, Stachowicz K, Black M, Parry M, Burke C, Meier S, Amer P. 2018. Genotype by environment interactions in fertility traits in New Zealand dairy cows. *Journal of Dairy Science* 101: 10991-11003.
- DairyNZ. 2020a. NZ Dairy Statistics 2019-20. Retrieved from 'https://d1r5hvvxe7dolz.cloudfront.net/media/documents/NZ_Dairy_Statistics_2019-20_WEB_FINAL.pdf'; 56. [Date accessed: 13 December 2021].
- DairyNZ. 2020. Your index - Your animal evaluation system. Retrieved from 'http://clarkiescourses.weebly.com/uploads/2/6/0/9/26099296/breeding_worth.pdf'. [Date accessed: 22 December 2021].
- DairyNZ. 2020b. New Zealand Dairy Statistics 2020-21. Retrieved from 'https://d1r5hvvxe7dolz.cloudfront.net/media/documents/NZ_Dairy_Statistics_2020-21.pdf'; 4-5. [Date accessed: 13 December 2021].
- DCANZ. 2021. About the New Zealand dairy industry. Retrieved from [https://www.dcanz.com/about-the-nz-dairy-industry/#:~:text=In%2030%20years%2C%20dairy%20exports,%25%20in%20four%20of%20those\).](https://www.dcanz.com/about-the-nz-dairy-industry/#:~:text=In%2030%20years%2C%20dairy%20exports,%25%20in%20four%20of%20those).) [Date accessed: 12 December 2021].

- Dezetter C, Leclerc H, Mattalia S, Barbat A, Boichard D, Ducrocq V. 2015. Inbreeding and crossbreeding parameters for production and fertility traits in Holstein, Montbéliarde, and Normande cows. *Journal of Dairy Science* 98: 4904-4913.
- Doekes HP, Veerkamp RF, Bijma P, de Jong G, Hiemstra SJ, Windig JJ. 2019. Inbreeding depression due to recent and ancient inbreeding in Dutch Holstein–Friesian dairy cattle. *Genetics selection evolution* 51: 1-16.
- Endres MI, Schwartzkopf-Genswein K. 2018. Overview of cattle production systems. *Advances in cattle welfare* 1-26.
- Garcia S, Holmes C. 1999. Effects of time of calving on the productivity of pasture-based dairy systems: A review. *New Zealand Journal of Agricultural Research* 42: 347-362.
- Gerssen-Gondelach SJ, Lauwerijssen RB, Havlík P, Herrero M, Valin H, Faaij AP, Wicke B. 2017. Intensification pathways for beef and dairy cattle production systems: Impacts on GHG emissions, land occupation and land use change. *Agriculture, Ecosystems & Environment* 240: 135-147.
- González-Recio O, De Maturana EL, Gutiérrez J. 2007. Inbreeding depression on female fertility and calving ease in Spanish dairy cattle. *Journal of Dairy Science* 90: 5744-5752.
- Granleese T, Clark SA, Swan AA, van der Werf JH. 2015. Increased genetic gains in sheep, beef and dairy breeding programs from using female reproductive technologies combined with optimal contribution selection and genomic breeding values. *Genetics Selection Evolution* 47: 1-13.
- Grosshans T, Xu Z, Burton L, Johnson D, Macmillan K. 1997. Performance and genetic parameters for fertility of seasonal dairy cows in New Zealand. *Livestock Production Science* 51: 41-51.
- Gutiérrez-Reinoso MA, Aponte PM, Cabezas J, Rodríguez-Alvarez L, García-Herreros M. 2020. Genomic evaluation of primiparous high-producing dairy cows: Inbreeding effects on genotypic and phenotypic production–reproductive traits. *Animals* 10: 1704.

- Harris B. 2005. Breeding dairy cows for the future in New Zealand. *New Zealand Veterinary Journal* 53: 384-379.
- Harris BL. 1998. Breeding dairy cattle for economic efficiency: a New Zealand pasture-based system. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production*: 383-386.
- Henderson CR. 1976. A simple method for computing the inverse of a numerator relationship matrix used in prediction of breeding values. *Biometrics* volume: 69-83.
- Keller MC, Visscher PM, Goddard ME. 2011. Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. *Genetics* 189: 237-249.
- Lembeye F, López-Villalobos N, Burke J, Davis S. 2016. Breed and heterosis effects for milk yield traits at different production levels, lactation number and milking frequencies. *New Zealand Journal of Agricultural Research* 59: 156-164.
- LIC. 2021. Sire proving scheme. Retrieved from '<https://www.lic.co.nz/products-and-services/artificial-breeding/sire-proving-scheme/>'. [Date accessed: 11 December 2021].
- Maiwashe A, Nephawe K, Theron H. 2008. Estimates of genetic parameters and effect of inbreeding on milk yield and composition in South African Jersey cows. *South African Journal of Animal Science* 38: 119-125.
- Martikainen K, Koivula M, Uimari P. 2020. Identification of runs of homozygosity affecting female fertility and milk production traits in Finnish Ayrshire cattle. *Scientific Reports* 10: 1-9.
- McParland S, Kearney J, Rath M, Berry D. 2007. Inbreeding effects on milk production, calving performance, fertility, and conformation in Irish Holstein-Friesians. *Journal of Dairy Science* 90: 4411-4419.
- Meuwissen THE. 1987. Maximizing the response of selection with a predefined rate of inbreeding. *Journal of Animal Science* 75: 934-940.

- Nietlisbach P, Keller LF, Camenisch G, Guillaume F, Arcese P, Reid JM, Postma E. 2017. Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. *Proceedings of the Royal Society B: Biological Sciences* 284: 2016-2763.
- Pawson E, Perkins HC. 2017. New Zealand going global: The emerging relationships economy. *Asia Pacific Viewpoint* 58: 257-272.
- Penasa M, Dal Zotto R, Cassandro M, López-Villalobos N, Evans RD, Cromie AR. 2010. Crossbreeding effects on milk yield traits and calving interval in spring-calving dairy cows. *Journal of Animal Breeding and Genetics* 127: 300-307.
- Pitt CJ. 2007. Lowering sperm dose rates in frozen semen for bovine artificial breeding. The University of Waikato.
- Pryce JE, Haile-Mariam M, Goddard ME, Hayes BJ. 2014. Identification of genomic regions associated with inbreeding depression in Holstein and Jersey dairy cattle. *Genetics selection evolution* 46: 1-14.
- Quaas R. 1976. Computing the diagonal elements and inverse of a large numerator relationship matrix. *Biometrics* volume: 949-953.
- Rebelato AB, Caetano AR. 2018. Runs of homozygosity for autozygosity estimation and genomic analysis in production animals. *Pesquisa Agropecuária Brasileira* 53: 975-984.
- Rendel JM, Robertson A. 1950. Estimation of genetic gain in milk yield by selection in a close herd of dairy cattle. *Journal of Genetics* 50: 1-8.
- Rokouei M, Torshizi RV, Shahrabak MM, Sargolzaei M, Sørensen A. 2010. Monitoring inbreeding trends and inbreeding depression for economically important traits of Holstein cattle in Iran. *Journal of Dairy Science* 93: 3294-3302.
- Smith LA, Cassell B, Pearson R. 1998. The effects of inbreeding on the lifetime performance of dairy cattle. *Journal of Dairy Science* 81: 2729-2737.
- Sneddon N, Lopez-Villalobos N, Hickson R, Shalloo L, Garrick D. 2015. Estimation of crossbreeding effects on yields of dairy products and value of milk processed in

different product portfolios. Proceedings of the New Zealand Society of Animal Production Volume: 48-53.

Temesgen MY, Assen AA, Gizaw TT, Minalu BA, Mersha AY. 2022. Factors affecting calving to conception interval (days open) in dairy cows located at Dessie and Kombolcha towns, Ethiopia. PloS one 17: e0264029.

VanRaden PM. 2008. Efficient methods to compute genomic predictions. Journal of Dairy Science 91: 4414-4423.

Verkerk G. 2003. Pasture-based dairying: challenges and rewards for New Zealand producers. Theriogenology 59: 553-561.

Villanueva B, Fernández A, Saura M, Caballero A, Fernández J, Morales-González E, Toro MA, Pong-Wong R. 2021. The value of genomic relationship matrices to estimate levels of inbreeding. Genetics Selection Evolution 53: 1-17.

Woolford M, Phillips D, Copeman P. 1982. New concepts in milking management and milking machines [cattle, New Zealand]. Proceedings of the Ruakura Farmers' Conference.

Wright S. 1922. Coefficients of inbreeding and relationship. The American Naturalist 56: 330-338.