Genetic analysis of milking temperament and its relationships with milk production traits in South African holstein cattle.

GENETIC ANALYSIS OF MILKING TEMPERAMENT AND ITS RELATIONSHIPS WITH MILK PRODUCTION TRAITS IN SOUTH AFRICAN HOLSTEIN CATTLE

By

THULI TINTSWALO SIWELE

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Supervisor	**:**	Prof. C.B. Banga
Co-supervisor	**:**	Prof. B.J. Mtileni
Co-supervisor	**:**	Prof. K.A. Nephawe

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DECLARATION

“I hereby declare that the dissertation submitted for the degree Master of Agriculture (Animal sciences) at Tshwane University of Technology is my own work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references”.

Candidate: T.T Siwele……..……

Date: 09-01-2024…….……...

Supervisor: Prof C.B. Banga……...

Date 09-01-2024…..……...

Co-supervisor: Prof B.J. Mtileni.……...

Date 09-01-2024……..……...

Co-supervisor: Prof K.A. Nephawe……...

Date 09-01-2024……..……....

DEDICATIONS

This dissertation is dedicated to my mother Jane Mashiane, my children Ntsako and Vutomi and to all my siblings' (David, Fridah, Phumzile, Siza and Tshami) and the entire family thank you for your unwavering love and support.

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ABSTRACT

Milking temperament (MT) is an important functional trait in dairy cattle, as it influences herd profitability, human and animal welfare. There is, however, limited knowledge such as genetic parameter estimates on MT in South African Holstein cattle, which makes it difficult to improve the trait genetically. The aim of this study was to estimate genetic parameters for MT and its relationships with milk production traits (milk yield, fat and protein) in South African Holstein cattle. Data consisted of MT scores on 2 844 cows from 16 herds participating in the National Milk Recording and Improvement Scheme, recorded from September 2020 to November 2021. Corresponding test-day records of milk yield and composition, and pedigree information, were obtained from the Integrated Registration and Genetic Information System of South Africa. Analysis of variance was performed using the General Linear Models procedure of the SAS software, to determine non-genetic factors affecting MT and milk production traits. Repeatability of MT scores was estimated using the variance components procedure of the SAS software. Genetic parameter estimates were obtained by linear animal models, using the ASReml software. Herd test-day, age of cow at calving ($P < 0.0001$) and lactation stage ($P < 0.05$) had significant effects on MT. The repeatability estimate for MT scores was moderate (0.47), while the estimate of heritability was low (0.05±0.04). Heritability estimates for milk production traits were low to moderate, ranging from 0.11±0.05 for milk yield to 0.24±0.06 for protein %. Milking temperament had a moderate and favourable genetic correlation (0.60±0.35) with milk yield. In contrast, low and unfavourable correlations were observed for MT with fat % (-0.12±0.24) and protein % (-0.30±0.32). Phenotypic correlations were also positive for MT with milk yield (0.25±0.02) and negative with fat (-0.06±0.03) and protein % (-0.04±0.02). There was no discernible genetic trend for MT, for animals born from 2009

to 2019, and a marginal overall decline was observed over the period. These results indicate reasonable consistence in the assessment of MT in the current study, and suggest that there is scope to improve MT through selection, using multi-trait models including milk yield.

Key Words: dairy cows, milking behaviour, genetic improvement, milk composition, welfare

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ACC	Age of cow at calving
ANOVA	Analysis of Variance
ARC	Agriculture Research Council
CV	Coefficient of variation
DARD	Department of Agricultural Rural Development
DIM	Days in milk
GLM	General Linear Model
h^2	Heritability
HTD	Herd-test-day
INTERGIS	Integrated Registration and Genetic Information System
kg	Kilogram
LS	Stage of lactation
NMRIS	National Milk Recording and Improvement Scheme
MPO	Milk Production Organisation
MT	Milking Temperament
MY	Milk yield
R^2	Repeatability
r_g	Genetic correlation;
r_p	Phenotypic correlation
SE	Standard Error
SA	South Africa
SAS	Statistical Analysis Software
SASAS	South African Society for Animal Science
SD	Standard Deviation

SCC	Somatic cell count
TUT	Tshwane university of Technology
TUT	Tshwane university of Technology
%	Percent

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Milking temperament is a measure of the response or reactiveness of a cow to the milking process. It expresses the level of comfort or stress experienced by the cow, as indicated by its behavioural reaction or level of nervousness and, therefore, is related to animal welfare (Wenzel *et al*., 2003; Szentleleki *et al*., 2015). Extreme reactiveness (i.e. poor temperament) can threaten the safety of other animals and that of the animal handlers; hence, temperament influences both animal and human welfare (Costantini *et al*., 2008; Mincu *et al*., 2021). Besides its importance as a welfare-related trait, milking temperament has large implications on herd profitability as it has been found to be associated with cow performance indicators such as milk yield and compositional quality (Cziszter *et al.,* 2016), performance in automated milking systems (Wethal and Heringstad, 2019), milking speed (Jacobsen *et al*., 2009; Kramer *et al.,* 2013; Agravat *et al*., 2023), reproduction (Sewalem *et al.,* 2011; Cziszter *et al.,* 2016), udder health (Santos *et al.,* 2018) and survivability (Chang *et al.,* 2019).

Although there is generally no consensus among researchers on the relationship between milking temperament and milk production (Marcal-Pedroza *et al*., 2023), most studies have found that highly reactive or nervous animals tend to produce less milk (Sziszter, 2003; Muller and Von Keyserlingk, 2006; Quist *et al*., 2008; Abdel-Hamid *et al*., 2017; Santos *et al.,* 2018; Priyam *et al*., 2023). The negative effects of high reactiveness on milk yield may be attributable to the fact that stress causes elevated levels of cortisol and endogenous opioids in plasma, coupled with lower concentrations of oxytocin (Chang *et al*., 2020). Stress due to fearfulness during milking has also been reported to influence milk composition by reducing the solid

constituents (Muller and Von Keyserlingk, 2006; Priyam *et al*., 2023). This could be due to the metabolic disorders caused by the stress experienced by the cow, which compromises milk quality (Cooke *et al*., 2017). Prolonged milking of cows with poor temperament also has negative effects on herd profitability, due to increased costs of labour and electricity (Berry *et al*., 2005; Sewalem *et al*., 2010; Abdel-Hamid *et al*., 2017). In automated milking systems, poor temperament has been documented to negatively affect the milking process through increased numbers of rejected or incomplete milkings, kickoffs of clusters and unallocated teats (Wethal and Heringstad, 2019).

Stress due to poor cow temperament has also been reported to be detrimental to health and reproductive performance (Santos *et al*., 2015; Brown and Vosloo, 2017; Brandao and Cooke, 2021; Mincu *et al*., 2021). Increased levels of neutrophils, cortisol and glucose have been observed in reactive than calm animals, as an indicator of elevated stress (Hulbert *et al*., 2011). For example, Mincu *et al*. (2021) noted that calmer animals may be less susceptible to metabolic diseases than their nervous counterparts. Furthermore, Antanaitis *et al*. (2021) found higher pregnant rates in European Holstein cows with favourable temperament than those with poor temperament. Higher pregnancy rates were also reported for calmer animals (Kasimanickam *et al*., 2014). This may be attributed to high stress levels inhibiting the animal's response to the administration of Gonadotropin-releasing hormone (GnRH), which interferes with the Luteinizing hormone (LH) peak and ovulation (Cooke *et al*., 2009; Fernandez-Novo *et al*., 2020). Moreover, agitated cows have been found to be more associated with reproductive disorders such as retained placenta and metritis (Chebel *et al*., 2016).

Milking temperament has an impact on herd replacement costs and lifetime production, as animals with poor temperament are at a higher risk of being culled early (Kenttamies *et al*., 2006; Sewalem *et al*., 2010; Kalinska and Slosaraz, 2016). For example, Sewalem *et al*. (2010) noted that 26% of involuntary culling in Canadian Holstein herds was due to poor milking temperament.

Dairy breeding objectives worldwide are increasingly being broadened to incorporate functional and welfare-related traits that received little attention in the past (Miglior *et al.,* 2017). In addition to traits such as those related to reproduction and health, there is a growing interest in management or workability traits such as milking temperament, due to their economic importance and association with welfare and ease of management (Szymik *et al*., 2015; Costilla *et al*., 2020).

Holstein is the major dairy cattle breed in South Africa, and comprises about 46% of the milk recorded population (DARD, 2019/20; NMRIS, 2022). Extensive research has been conducted and routine genetic evaluation is conducted for traits related to production, conformation, udder health (somatic cell score) and reproduction in South African Holstein cattle (Makgahlela *et al.*, 2007; Dube *et al*., 2009; Banga *et al*., 2014; Tshilate *et al*., 2021; INTERBULL, 2022; Kgari *et al.*, 2022). Considerable genetic improvement has been attained in this population for production traits (Ramatsoma *et al*., 2014). There has, however, been limited attention on workability or functional traits such as milking temperament. Few studies have been conducted on these traits in South African dairy cattle (Dodzi and Muchenje, 2011; Tshilate *et al*., 2021; Mpisana *et al*., 2021). It is important to study the genetic attributes of functional traits, in order to facilitate their inclusion in the genetic evaluation programme and breeding objective and, therefore, keep pace with global developments and remain competitive.

1.2. Problem Statement

Aggressiveness, or excessive fear during milking, is an undesirable temperament characteristic in dairy cattle. It has been found to be detrimental to cow performance and herd profitability and has negative implications on animal and human welfare. Therefore, milking temperament is an important trait, and should be included in dairy cattle selection objectives. There is growing interest in genetic selection to improve milking temperament in dairy cattle breeding programs worldwide (INTERBULL, 2022). Although South Africa has a sound genetic evaluation program, milking temperament and other functional traits are not included in the selection programme, and there has been limited attention to these traits. It is therefore important to incorporate these traits in the genetic evaluation program, in order to keep pace with global developments and ensure a sustainable dairy industry. However, estimates of genetic parameters, which are essential for genetic evaluation, are not available for milking temperament in South African dairy cattle.

1.3. Justification

Milking temperament is an important trait in dairy cattle, due to its effects on herd profitability, human and animal welfare. Accordingly, it is receiving growing attention and is increasingly being included in dairy breeding objectives worldwide. Currently, milking temperament is not included in the national genetic evaluation programme for South African dairy cattle. Genetic correlations between milking temperament and milk production traits in the South African Holstein cattle population can be used to improve accuracy of genetic evaluation for milking temperament through multi-trait analysis. Results of the current study provide the means for achieving accurate selection for

milking temperament in the South African Holstein cattle population. This will contribute towards improved human and animal welfare and profitability in South African dairy herds.

1.4. Aim and Objectives

The aim of the study was to evaluate non-genetic and genetic influences on milking temperament, and estimate its relationships with production traits (milk yield, fat and protein percentage) in South African Holstein cattle.

The specific objectives were to:

i. Estimate the repeatability of milking temperament scores.
ii. Identify non-genetic (environmental) factors influencing milking temperament in South African Holstein cattle.
iii. Estimate heritabilities and genetic and phenotypic correlations between milking temperament and daily milk yield, fat and protein percent in South African Holstein cattle.

1.5. Hypotheses

The null hypotheses tested in South African Holstein cattle were:

i. Milking temperament scores are not repeatable.
ii. There are no non-genetic factors influencing milking temperament.
iv. There is no genetic (co)variation among milking temperament and daily milk yield, fat and protein percent in South African Holstein cattle.

1.6. Research outputs and conference contribution

SIWELE, T.T., DUBE, B., MTILENI, B.J., NEPHAWE, K.A., MADILINDI, M.A. & BANGA, C.B. 2021. Non-genetic influences on milking temperament in South African Holstein cattle. 52nd Annual SASAS Congress (Online) Poster.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

An overview of the South African dairy industry is presented in this chapter, together with background information on the Holstein cattle breed of South Africa, and a discussion of the importance of milking temperament. Additionally, literature on non-genetic (environmental) and genetic influences on milking temperament, and genetic and phenotypic correlations between milking temperament and milk production traits are reviewed.

2.2 The South African dairy industry

Dairy farming supports the livelihoods more than a billion people worldwide, and milk provides a source of many vital nutrients that are essential to sustain the growing world human population (Nguyen *et al*., 2023). Increased global milk production is, therefore, key to ensuring socio-economic and food security worldwide. In order to meet the growing demand for milk as well as remain a sustainable agricultural sector, the dairy industry worldwide needs to improve efficiency while conserving the environment and ensuring good animal welfare (Tucker, 2015; Nguyen *et al*., 2023).

In South Africa, the dairy industry is the fourth largest agricultural sector, and currently it accounts for approximately R4 584 billion gross domestic product (GDP) of the South African agricultural sector (Milk SA, 2023). The industry contributes significantly to the South African economy by providing employment, food security and export revenues (Milk SA, 2023). In the year 2019/2020, there were around 1200 farms in South Africa, which generated approximately 60,000 jobs (SA Yearbook, 2019/20). Additionally, unlike most other agricultural activities, dairy production provides year-round

employment opportunities for many individuals (DAFF, 2012). In 2022 alone, the South African dairy industry exported 52 000 tons of dairy products, thus bringing in much-needed foreign currency to the country (Milk SA, 2023).

Recent trends show consistently increasing national milk production in South Africa, despite a concurrent decline in the number of large-scale commercial dairy farmers. A 9.5% decrease in commercial dairy herds (from 984 to 891) was recorded between 2022 and 2023, while national milk production increased by 2.9 % to 334,900 tons from 2017 to 2022 (Milk SA, 2023), it can be attributed to the dairy genetic breeding improvement programme which focus on production traits, mainly increased milk yield as the primary aim for genetic progress (Hansen, 2000; Brotherstone and Goddard, 2005; Erasmus and Van Marle-Koster, 2021). Furthermore, it has been reported that average milk yield per animal has increased significantly over the years (Mekonnen *et al*., 2019), resulting to fewer cows required per unit of milk produced (Balmfort *et al*., 2018).

Although milk is produced throughout South Africa, most of the production is concentrated in the coastal areas (Eastern Cape, Western Cape and KwaZulu-Natal), because of the warm temperatures and abundant rainfall, which promote good pasture growth (SAWS, 2021; Milk SA, 2023). Approximately 85.8% of the milk is produced in the coastal areas, with the Western Cape being the highest producing province (29.3%), followed by the Eastern Cape (28.5%), and KwaZulu-Natal (28.0%) (MPO, 2022; Milk SA, 2023).

The South African dairy industry consists of several organizations that fulfill various roles, and these include Milk South Africa (Milk SA), Milk producers' Organization (MPO), South African Milk Processors' Organisation (SAMPRO) and Agricultural

Research Council (ARC) (SA Yearbook, 2019/20). Milk SA is the governing body of the primary and secondary dairy sectors in South Africa, managing the dairy industry in the country, while the MPO represents the interests of unprocessed milk producers and SAMPRO is a representative organization of the milk processors and dairy products manufactures in South Africa (Milk SA, 2022). The ARC manages the National Milk Recording and Improvement Scheme (NMRIS), which aims to improve production and economic efficiency of dairy herds (Van Den Bergh, 2010). It also offers technical support to dairy farmers to help them in making sound management decisions (SA Yearbook, 2019/20). The data collected from the farms through the NMRIS is stored in the national database known as the integrated registration and genetic information system (INTERGIS) of South Africa (Intergis, 2000; SA Yearbook, 2019/20). The number of commercial farmers participating in the NMRIS has been decreasing because more farmers are moving towards automatic milking systems and recording (van Marle-Koster and Visser, 2018).

2.3 Dairy cattle genetic improvement in South Africa

Routine genetic evaluation in South African dairy cattle is carried out for traits related to production (milk yield, fat and protein), reproduction (calving interval and age at first calving), udder health (somatic cell count) and conformation (Imbayarwo-Chikosi *et al*., 2017). Many years of genetic selection, using estimated breeding values, have led to significant changes in production and conformation traits (Ramatsoma *et al*., 2014). Functional traits were, however, largely ignored in the national selection programme in the past (Imbayarwo-Chikosi *et al*., 2017).

2.4 Holstein breed of South Africa

There are four main dairy cattle breeds in South Africa, namely Holstein, Jersey, Guernsey and Ayrshire (SA Yearbook, 2019/20). Holstein is the primary dairy cattle breed in South Africa (Banga *et al*., 2014). The breed originated in the provinces of northern Holland and western Friesland in the Netherlands, and was introduced into South Africa in 1912 (Poena van Niekerk, 2019). Holstein cattle have desirable phenotypic characteristics, such as their distinctive large size, and sharply defined black and white spotted markings (red and white in some cattle) (Poena van Niekerk, 2019). They have high milking ability (average milk yield of 28 litres per day and 8,550 kg per lactation), and weigh around 550-750 kg (Poena van Niekerk, 2019). Although their milk is low in fat content (2.5 to 4.3%), the Holstein is the highest milk yielding dairy cattle breed (Yoo *et al*., 2019; (Poena van Niekerk, 2019). Holstein cows have a stress-resistant temperament; they are not solitary animals; they exhibit a herd mentality, and they adapt well to all management and utilization systems (Poena van Niekerk, 2019).

2.5 Functional traits in dairy cattle

Functional traits are those characteristics of an animal that influence its functionality, and are related to production costs (Boettcher, 2005). Examples of such traits include health, fertility, feed efficiency, milkability, and milking temperament. Previous research on dairy cattle in South Africa has mainly focused on traits related to milk production, udder health, conformation and reproduction (e.g., Dube *et al.,* 2009; Banga and Maiwashe, 2014; Erasmus and van Marle-Koster, 2021; Kgari *et al.,* 2022). Limited studies have been carried out on functional traits such as milkability and feed

efficiency (Tshilate *et al*., 2021; Madilindi *et al*., 2023). Globally, there has however been increased attention on functional traits such as those associated with carbon emission, feed efficiency, and animal health and welfare (Miglior *et al*., 2017; van Marle-Koster and Visser, 2018; Madilindi *et al.*, 2022, 2023). There has also been growing interest on workability traits such as milking temperament, due to their effect on herd profitability and animal welfare (Egger-Danner *et al*., 2015; Chesnais *et al*., 2016).

2.5.1 Importance of milking temperament

Milking temperament refers to the type and degree of reaction of the animal to the whole milking procedure. It reflects the level of comfort or stress experienced by the cow, as indicated by its behavioural reaction or level of nervousness and, therefore, is related to animal welfare (Wenzel *et al*., 2003; Szentleleki *et al*., 2015). Animals with favourable (calm) temperament are also easier to handle, feed, milk and transport (Santos *et al*., 2018; Costilla *et al*., 2020; Jaskowski *et al*., 2023). Extreme reactiveness (i.e. poor temperament) can threaten the safety of other animals and that of the animal handlers; hence, temperament influences both animal and human welfare (Mincu *et al*., 2021; Smolinger and Skorjanc, 2021). Besides its importance as a welfare-related trait, milking temperament has large implications on herd profitability as it has been found to be associated with cow performance indicators such as milk yield and compositional quality (Cziszter *et al.,* 2016), udder health (Santos *et al.,* 2018), survivability (Chang *et al.,* 2019), reproduction (Sewalem *et al.,* 2011; Cziszter *et al.,* 2016), milking speed (Jacobsen *et al*., 2009; Kramer *et al.,* 2013; Agravat *et al*., 2023) and performance in automated milking systems (Wethal and Heringstad, 2019).

2.5.1.1 Effects of milking temperament on milk production traits

Milking temperament has been widely documented to influence milk yield and compositional quality (Kasimanickam *et al*., 2018; Chang *et al*., 2020; Mincu *et al*., 2021; Marcal-Pedroza *et al*., 2023). Several studies have found calm cows to produce significantly more milk than aggressive and nervous ones (Haskell *et al.,* 2014; Sutherland and Dowling, 2014; Cziszter *et al*., 2016; Abdel-Hamid *et al*., 2017; Mincu *et al*., 2021; Morales-Pineyrua *et al*., 2022). For example, in a study on Egyptian Friesian cattle, calm cows produced about 11 kg of milk per day more than nervous cows, and about 19 kg of milk per day more than aggressive cows (Abdel-Hamid *et al*., 2017). Similarly, Neja *et al*. (2015) reported that calm cows yielded approximately 329 kg more milk than aggressive cows per lactation in Polish Holstein-Friesian cattle. Another study on Ukrainian Holstein cattle also noted that calm cows produced about 275 kg more milk per lactation than active ones (Polupan *et al*., 2021). Nervousness during milking induces stress which has been reported to affect milk composition by lowering solid components (Muller and Von Keyserlingk, 2006; Priyam *et al*., 2023). Studies on Bulgarian Holstein-Friesian cows have, however, reported higher milk yields for nervous cows compared to those with calm milking temperament (Gergovska *et al.*, 2012; Gergovska *et al.*, 2014).

Calm temperament appears to also favour desirable compositional quality of milk. For example, Marcal-Pedroza *et al*. (2023) reported a significantly higher protein percent per day in calm than aggressive cows, in Brazilian Holstein-Gyr cattle. Similar results were obtained in Ukrainian Holstein cattle by Polupan *et al*. (2021), who recorded 9 kg more protein yield per lactation in calm than active cows. Radu *et al*. (2023) also reported that calm cows produced more protein yield compared to nervous ones, in a study on Romanian Simmental dual-purpose cows. The increase in protein production

could partly be due to the lower stress levels in calm cows resulting in favourable metabolic processes for protein synthesis (Muller and Von Keyserlingk, 2006; Cooke *et al*., 2017; Priyam *et al*., 2023). Contrary to these findings, however, nervous/aggressive cows were found to produce higher fat and protein percent than calm cows in Romanian Simmental dual-purpose cattle (Cziszter *et al.*, 2016). Lower milk yield in nervous cows may result in higher fat and protein content, given the established negative relationship between milk yield and composition (Cziszter, 2003; Quist *et al.,* 2008; Santos *et al.,* 2018).

2.5.1.2 Effects of milking temperament on cow longevity

Cows with poor milking temperament tend to be culled prematurely, resulting in reduced longevity and, consequently, lower herd profitability. For example, a culling rate of 26% was reportedly associated with nervousness and aggressiveness of cows in the Canadian Holstein cattle population (Sewalem *et al.*, 2010). Similarly, Neja *et al*. (2015) noted that aggressive cows were culled at a rate of 27.41 % more than calm ones in Polish Holstein-Friesian cattle. Haile-Mariam *et al*. (2023) found a correlation of -0.19 between temperament and slaughter rate in Australian Holstein cattle, indicating an increase in risk of culling for cows with poor temperament.

2.5.1.3 Effects of milking temperament on reproduction

Results from many studies suggest that there may be an association between milking temperament and reproductive performance of cattle (Sewalem *et al.,* 2011; Cooke e*t al*., 2017; Sawa *et al*., 2017; Antanaitis *et al*., 2021; Mincu *et al*., 2021). For instance, Polish Holstein-Friesian cows with calm temperament were reported to have favourable fertility rate (Sawa *et al*., 2017). In Brazil, Cooke *et al*. (2017) reported that

excitable *Bos indicus* beef cows had reduced reproductive performance. These results were supported by more recent research which found higher pregnant rates in calmer temperament animals than those with poor (nervous) temperament (Kasimanickam *et al*., 2018; Antanaitis *et al*., 2021). Reproductive disorders, such as retained placenta and metritis, have also been linked to poor temperament in cows (Chebel *et al*., 2016). Contrary to all these findings, however, Cziszter *et al.* (2016) found no effect of temperament on reproductive performance in Romanian Simmental dual-purpose cattle. Detrimental effects of poor temperament on reproductive performance could be due to the high level of stress in nervous cows interfering with the peak release of Luteinizing hormone (LH) and ovulation (Fernandez-Novo *et al*., 2020; Brandao and Cooke, 2021).

2.5.1.4 Effects of milking temperament on health and welfare

Poor milking temperament has been generally associated with impaired health and welfare (Costantini *et al*., 2008; Hulbert *et al*., 2011; Cooke *et al*., 2017; Santos *et al.,* 2018; Chang *et al*., 2020; Mincu *et al*., 2021). A nervous response to the milking process is indicative of elevated stress levels. For example, increased levels of cortisol and oxytocin concentration were found in Brazilian Holstein-Gyr cattle with poor temperament (Marcal-Pedroza *et al*., 2023). Stress indicators such as heart rate level and cortisol concentration were similarly reported to be significantly higher in nervous cows during milking than in calm animals (Gygax *et al*., 2006; Gygax *et al*., 2008). Cortisol hormone is mainly associated with physiological and neuroendocrine stress response (Cockrem, 2013; Cooke, 2014). Orban *et al*. (2011) reported that calmer cows had lower somatic cell count (SCC) of $176.07x10^3/cm^3$ compared to nervous

counterparts in Hungarian Holstein-Friesian cattle, which leads to reduced milk yield and compositional quality and increased udder inflammation.

2.5.2 Measurement of milking temperament

Large quantities of reliable data are required to achieve accurate selection for any trait in a population. However, recording of milking temperament is labour-intensive, and difficult to carry out in large herds. It may be done by screening for aggressiveness or excessive fear during milking or handling (Haskell *et al*., 2014). Temperament can also be measured during stressful situations by observing the behavioural response of the animal and giving it a score (Chang *et al*., 2020). Milking temperament scores are commonly recorded on scales of 1 to 3, 1 to 4, 1 to 5, 1 to 9, or 1 to 50 (Sewalem *et al.,* 2010; Sutherland and Dowling, 2014; Stephansen *et al.,* 2018). Table 2.1 presents a description of the scores that are commonly used when assessing milking temperament through this approach (Gergovska *et al*., 2012). This method is easy to apply in small herds; however, it is difficult to use in large dairy cattle operations as it is labour-intensive (Chang *et al.,* 2020).

Table 2.1: Description of scores for milking temperament (Gergovska *et al*., 2012)

Class	Definition	Description of the behavior
1	Very nervous/ Aggressive	Very restless during preparation and milking process, the animals showed signs of lifting their leg, kicking the milking machines and the farm workers, slashes the tail.
2	Nervous	Perceived restless during preparation and milking process, the animal startled when humans approach.
3	Medium	They were calm but they move a lot and lift legs during preparation and milking process.
4	Calm	Stand calm on the bedding the animal did not show any type of restlessness during preparation and milking process, slash the tail.
5	Very calm	Never showed restlessness, the animals were fully calm and obedient during preparation and milking processes.

Novel and more efficient technologies such as video imaging, activity collars, pedometers, and metabolite profiling, which can standardize temperament evaluations across farms, have been developed (Chang *et al.,* 2020). Investment in such technologies will make it possible to achieve more accurate genetic evaluation of milking temperament through large scale recording across herds. Activity monitors can be used to detect animal behavioural and physiological activities, while video imaging methods have made it possible to better assess the temperament of dairy cattle (Chang *et al*., 2020). Biomarkers such as heart rate and cortisol level, which can be linked to stress level, have been associated with temperament traits (Chang *et al*., 2020; Smolinger and Skorjanc, 2021; Jaskowski *et al*., 2023). The advantage of these methods is that they do not interfere with herd routine procedures and they generate large quantities of repeated records which enable better estimation of breeding values and, consequently, more accurate selection (Chang *et al*., 2020).

2.7 Genetic improvement of milking temperament

Milking temperament is now increasingly included in dairy breeding programmes worldwide (e.g., United Kingdom, Denmark, Sweden, France, Norway) (Byrne et al., 2016; Chang et al., 2020; INTERBULL, 2022). In South Africa, there is, however, limited knowledge on milking temperament and no efforts have been made to include the trait in the national recording and genetic improvement programmes (INTERBULL, 2022). Therefore, in order to develop models for genetic evaluation of milking temperament so as to include it in the selection objective, it is imperative to identify environmental factors that need to be fitted in such models as well as estimate genetic parameters for milking temperament in the South African Holstein population.

2.5.3 Environmental influences on milking temperament

Non-genetic or environmental factors are those effects that are not part of the genetic make-up of the animal and, therefore, are not passed down from one generation to the next (Nyamushamba *et al.,* 2013). These factors may cause substantial variation in phenotypic expression; hence, it is important to account for them when evaluating the genetic merit of individual animals. Knowledge and understanding of these factors can also assist in making sound management decisions (Amimo *et al*., 2007). Milking temperament may be influenced by factors such as herd, parity, lactation stage, age of cow, test day, year, season (Rensing and Ruten, 2005; Neja *et al*., 2015; Stephansen *et al*., 2018; Chang *et al*., 2020; Antanaitis *et al*., 2021; Marcal-Pedroza *et al*., 2023).

2.6.1 Herd

Herd is a primary source of variation in milking temperament (Jaskowski *et al*., 2023). Differences in milking temperament among herds are attributable to management factors such as animal handling, milking process and human approach, which may differ by herd (Uetake *et al*., 2004; Antanaitis *et al*., 2021). Milking personnel's relationship with the animals can significantly influence the level of stress or comfort experienced by cows during milking and, therefore, their milking temperament. For example, Bertenshaw and Rowlinson (2009) showed that bad attitude of milkers towards cows negatively affected milking temperament in United Kingdom dairy herds. On the other hand, a good relationship between milking personnel and the animals has been documented to result in calmer animal behaviour during handling (Breuer *et al*., 2000; Hemsworth and Coleman, 2011; Jaskowski *et al*., 2023). Therefore, herd effects need to be accounted for in genetic analysis models for milking temperament.

2.6.2 Parity

Parity has widely been reported as a significant source of variation in milking temperament (Sutherland and Huddart, 2012; Szentleleki *et al*., 2015; Chang *et al*., 2019; Morales-Pineyrua *et al*., 2022). First-parity cows have been found to be generally more nervous/aggressive during the milking process than their multiparous contemporaries. This may be attributed to the fact that primiparous cows tend to be more susceptible to stress caused by the noise of the milking machine that they are not used to, and they are also less accustomed to the general milking process and regrouping during early lactation (Sutherland and Huddart, 2012; Kovacs *et al*., 2013; Szentleleki *et al*., 2015; Chang *et al*., 2019; Mazer *et al*., 2020). Multiparous cows, on

the other hand, have been observed to be calmer during the milking process, most likely due to them being more accustomed to the milking process (Chang *et al*., 2019; Morales-Pineyrua *et al*., 2022). Parity is, thus, an important effect to consider in the genetic analysis of milking temperament.

2.6.3 Lactation stage

Milking temperament scores have been reported to vary significantly according to the stage of lactation, indicating the need to consider lactation stage in genetic analysis models (Gergovska *et al.,* 2012; Neja *et al.*, 2015; Antanaitis *et al.,* 2021). Most studies have found low temperament scores (nervousness/aggressiveness) to be more prevalent in early lactation, and to increase with stage of lactation (Gergovska *et al.,* 2012; Sawa *et al.*, 2017; Antanaitis *et al.,* 2021). Neja *et al.* (2015), on the contrary, found early lactating Polish Holstein-Friesian cows to be calm in temperament, while those in late lactation were aggressive. This may be attributed to the drop in milk production in late lactation causing cows to be uncomfortable and stressed during milking (Chebel *et al*., 2016; Proudfoot *et al*., 2018).

2.6.4 Age at calving

In concurrence with the observed effects of parity, many studies have noted improved milking temperament with increase in age at calving (Neja *et al*., 2015; Caetano *et al.*, 2017; Cielava *et al*., 2017; Almasri *et al*., 2020). Younger cows are naturally more nervous and, therefore, are expected to exhibit poorer temperament than older cows. Surprisingly, however, studies on Polish Holstein cattle have reported more aggressiveness by older cows during milking, compared to younger cows (Kalinska

and Slosarz, 2016; Karamfilov, 2022). There is, therefore, some inconsistence on the effects on age at calving on milking temperament.

2.6.5 Year of calving

Yearly variation in herd management and other environmental conditions may have an effect on milking temperament, as observed in some studies (Herve *et al*., 2007; Stephansen *et al*., 2018; Wethal and Heringstad, 2019). The introduction of unfamiliar conditions such automatic milking systems, increased noise, change of management groups or isolation may cause stress on cows and, therefore, negatively impact on their temperament.

2.6.6 Season of assessment

Milking temperament scores have been reported to significantly differ depending on the season of testing (Antanaitis *et al.,* 2021; Kumar and Mandal, 2023). Antanaitis *et al.* (2021) observed that, in Lithuanian Holstein cattle, more cows with calm temperament were recorded during the warm season (May to October), while more nervous cows were recorded in the cold season (November to April). On the other hand, Kumar and Mandal (2023) reported calmer milking temperament during the winter season compared to the rainy summer season in Indian Jersey crossbred cows. However, season/month of testing did not have significant effects on milking temperament of Brazilian Holstein-Gyr cattle (Marcal-Pedroza *et al*., 2023). It, therefore, appears that the effects of season of calving on milking temperament may depend on the region and/or breed.

2.6.7 Herd-test-day

Several studies on large data sets, using test-day models, have reported herd-test-day to be a significant source of variation in milking temperament (Rensing and Ruten, 2005; Szentleleki *et al.,* 2008; Sewalwem *et al*., 2011; Haskell *et al*., 2014; Wethal and Heringstad, 2019; Antanaitis *et al*., 2021). This may be attributable to variations in daily herd management practices within herds influencing milking temperament. Thus, herd-test-day is normally included as a contemporary group when milking temperament is analysed by test-day models.

2.8 Genetic parameters for milking temperament

Estimates of genetic parameters such as heritability, repeatability and genetic correlations are essential for carrying out genetic evaluation of a trait in a given population. Various analytical models have been applied to estimate genetic parameters for milking temperament in different dairy cattle populations (e.g., Abdel-Hamid *et al*., 2017; Chang and Wang, 2020; Szymik *et al.*, 2021; Batista-Taborda *et al*., 2023; Marcal-Pedroza *et al*., 2023). Estimates of heritability for milking temperament and genetic correlations between milking temperament and milk production traits were generally low in most of these studies. However, the genetic parameter estimates appear to vary with the method used to assess milking temperament and the analytical approach.

2.8.1 Repeatability of milking temperament scores

Repeatability, also known as the intraclass correlation coefficient, refers to the relative expression of the same trait at different times in the life of the same animal, or the

extent to which repeated measurements on an individual maintain a consistent value (Cilek and Sahin, 2009). It can also be described as a statistical measure of the consistency of repeated measurements, and is normally denoted by the letter ***r*** (Harper, 1994). Repeated measurements of the same animal could be identical in an ideal world. In practice, however, this is not the case as the observer unavoidably makes errors while measuring or scoring the animal (Barrett *et al*., 1989; Leverton, 1989). The trait being measured may also vary with time (Harper, 1994), due to changes in temporary environmental effects on the animal's phenotype. Since they can be used to predict future performance of the animal, repeatability estimates can assist in identifying which animals can be kept or culled in a herd (Cassell, 2009).

Some estimates of repeatability for milking temperament, from the literature, are summarized in Table 2.2. These estimates are generally moderate, ranging from 0.32 to 0.47 (Kramer *et al.,* 2013; Wethal and Heringstad, 2019; Polupan *et al*., 2021; Batista-Taborda *et al*., 2023). This indicates that repeated measures of milking temperament on a cow are likely to produce fairly similar scores. Differences in the analytical models applied and populations studied, as well the methods used to assess milking temperament, may partly explain the slight variation in these estimates.

Table 2.2: Published repeatability estimates for milking temperament

Reference	Country	Breed	Repeatability
Kramer *et al.* (2013)	Switzerland	Brown Swiss	0.32 ± 0.04
Wethal and Heringstad (2019)	Norway	Norwegian Red	0.43
Polupan *et al.* (2021)	Ukraine	Holstein	0.47
Batista-Taborda *et al.* (2023)	Brazil	Holstein-Gyr	0.38 ± 0.05 - 0.42 ± 0.02

2.8.2 Heritability of milking temperament

Heritability is an important parameter as it influences the accuracy of selection and

rate of genetic progress of a trait in the population. Table 2.3 presents some estimates of heritability for milking temperament in dairy cattle available in the literature. Most of these estimates are low and less than 0.20, although the range is quite large (0.03 to 0.36) (Kramer *et al.*, 2013; Stephansen *et al.*, 2018; Chang and Wang, 2020; Antanaitis *et al.*, 2021; Batista-Taborda *et al.*, 2023). Estimates based on subjective scores are generally low (0.03 to 0.14) (Cue *et al.*, 1996; Sewalem *et al.*, 2011; Rinell *et al.*, 2014; Chang and Wang, 2020; Antanaitis *et al.*, 2021; Batista-Taborda *et al.*, 2023) compared to those from automated machine connection data (0.26 to 0.36) (Stephansen *et al.*, 2018). This may suggest that subjective scoring of cows is associated with large error variance. The scoring level used, as well as analytical model applied, also seem to be a source of variation in estimates. These published estimates suggest that considerable genetic improvement in milking temperament can be achieved through selection; however, there is a need to improve assessment of the trait to minimise error of measurement.

Table 2.3: Heritability estimates for milking temperament from the literature

Country	Breed	MT score (Description)	Models	Heritability	References
New Zealand	Holstein	1 - 9 (Nervous - Calm)	SM	0.14	Cue *et al.* (1996)
Germany	Holstein	1 - 9 (Nervous - Calm)	RAM	0.07	Rensing and Ruten (2005)
Canada	Holstein	1 - 5 (Nervous - Calm)	LAM	0.13 ± 0.014	Sewalem *et al.* (2011)
Switzerland	Brown Swiss	1 - 4 (Nervous - Calm)	RAM	0.04 ± 0.04	Kramer *et al.* (2013)
Sweden	Holstein	1 - 9 (Nervous - Calm)	LAM	0.14	Rinell *et al.* (2014)
Danmark	Holstein	nApT	LAM	0.26 ± 0.02	Stephansen *et al.* (2018)
Danmark	Holstein	CT	LAM	0.36 ± 0.02	Stephansen *et al.* (2018)
Norway	Red dairy	0 or 1 (kick offs)	RAM	0.06 ± 0.01	Wethal and Heringstad (2019)
China	Holstein	1-3 (Calm - Aggressive)	GLMM	0.033 ± 0.017	Chang and Wang (2020)
Lithuania	Holstein	1 - 5 (Very calm - Very Aggressive)	LAM	0.044	Antanaitis *et al.* (2021)
Poland	Holstein-Friesian	1 - 3 (Calm - Aggressive)	LAM	0.08 ± 0.0063	Szymik *et al.* (2021)
Brazil	Holstein-Gyr	1 - 8 (Quiet - Agitated)	RAM	0.14 ± 0.03	Batista-Taborda *et al.* (2023)

MT=milking temperament; SM=sire model; RAM=repeatability animal model; LAM=linear animal model; GLMM=general linear mixed model; CT=Connection time; nApT=number of attachments per teat

2.8.3 Genetic and phenotypic correlations between milking temperament and milk production traits

Knowledge of genetic correlations may assist to establish the expected genetic change on a trait when selection is applied on other traits. Genetic correlations among traits in a population are also essential in the development of multi-trait selection objectives. Estimates of genetic correlations between milking temperament and milk production traits are scarce in the literature, and some of these are summarized in Table 2.4. These estimates generally range from low to moderate (Kruszynski *et al.*, 2013; Chang *et al.*, 2020; Antanaitis *et al.*, 2021). Chang *et al.* (2020) reported moderate genetic correlations, ranging from 0.27 to 0.42, for milking temperament with milk yield, protein and fat percent, in Chinese Holstein cattle. On the other hand, low estimates varying from 0.01 to 0.07 were obtained in Lithuanian (Antanaitis *et al.*, 2021) and Polish (Kruszynski *et al.,* 2013) Holstein cattle. Most of the genetic correlations were positive, suggesting that selection for good milking temperament (calm animals) may result in a correlated improvement in milk yield, protein and fat percent, and *vice versa*.

Literature on phenotypic correlations between milking temperament and milk production traits is scant. A recent study by Antanaitis *et al.* (2021) reported extremely low phenotypic correlations ranging from -0.024 between milking temperament and fat percent to 0.043 for milking temperament with milk yield, in Lithuanian Holstein cattle. It is difficult to make any inferences from such low correlation estimates, and based on a single study.

Table 2.4: Summary of genetic and phenotypic correlations between milking temperament and milk production traits from the literature

Traits	Genetic correlation	Phenotypic correlation	References
Milk yield	0.07	-	Kruszynski *et al.* (2013)
Protein percent	0.04	-	
Fat percent	0.01	-	
Milk yield	0.27	-	Chang *et al.* (2020)
Protein percent	0.42	-	
Fat percent	0.35	-	
Milk yield	-0.113	0.043	Antanaitis *et al.* (2021)
Protein percent	0.030	0.040	
Fat percent	0.011	-0.024	

2.9 Genetic trends for milking temperament

The genetic trend of a trait indicates genetic changes in a population over time, and can be obtained by regressing mean breeding values on birth year (Sahebhonar, 2007). Monitoring genetic trends assists in evaluating the effectiveness of breeding programs or the cumulative effects of selection decisions (Hintz and Van Vleck, 1978; Pezeshkian *et al*., 2016).

Sewalem *et al*. (2011) observed a gradual and uneven increase in genetic merit for milking temperament in Canadian Holstein bulls born from 1995 to 2005. Although milking temperament is not formally included in the selection objective for Canadian Holstein cattle, there has been intense selection for production traits for over a century (Sewalem *et al*., 2011). Cows with poor temperament are, however, selected against (Sewalem *et al.*, 2010), which may explain the gradual improvement in milking temperament in the population. The trend could also be a correlated response to selection for production or other traits in the selection objective. A negative genetic trend for milking temperament was, however, observed in the Polish Holstein Friesian population for the period 2001 to 2004 (Kruszynski *et al*., 2013).

2.10 Conclusions

Milking temperament is an important trait, since it influences herd profitability and welfare of both cows and humans; therefore, it should be included in dairy cattle breeding objectives. Calmness or good milking temperament has been largely associated with increased milk yield and compositional quality, improved cow longevity, higher conception rates, and better health and welfare. Non- genetic factors such as age at calving, herd, parity, and lactation stage have been documented to influence milking temperament; hence they should be accounted for in genetic analysis models. Repeatability estimates for milking temperament have been widely observed to be moderate, suggesting reasonable correlation between repeated scores on an animal. Low to moderate heritability estimates have been reported for milking temperament, which indicates potential for genetic improvement through selection. Genetic and phenotypic correlations between milking temperament and production traits in the literature are variable but mostly favourable. Selection for increased production may, therefore, improve milking temperament, and this is also inferable from genetic trends observed in some studies.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

Details of the data used in the current study, and the analyses carried out, are described in this chapter. The analyses involved computation of the repeatability of milking temperament scores, followed by an evaluation of the environmental factors influencing milking temperament, and estimation of genetic parameters among milking temperament, milk yield, and fat and protein percent. The genetic trend for milking temperament was also assessed, so as to determine if there have been any changes in the genetic merit for the trait over time.

3.2 Data

Milking temperament was assessed on milking cows from 16 Holstein herds participating in the National Milk Recording and Improvement Scheme, during the period September 2020 to November 2021. The herds were located in the South African provinces of Gauteng, Free State, Eastern Cape and KwaZulu-Natal. Four of the herds were scored 3 or 4 times at monthly intervals, and the rest were assessed only once. Milk production data on these cows, and their pedigree information, were obtained from the Integrated Registration and Genetic Information System (INTERGIS) of South Africa.

3.3 Assessment of milking temperament

Milking temperament (MT) of each cow was scored on a five-point-scale, according to Gergovska *et al.* (2012), as described in Table 3.1. All cows were assessed by one

person, and this was carried out during the afternoon milking for most of the herds (13). Scoring was done by observing the cows during milking, and due care was taken not to interfere with the milking process.

Table 3.1: Description of milking temperament scoring system (Gergovska *et al.,* 2012)

Score	Definition	Description of the behavior
1	Very nervous	Very restless during milking process with kicking and lifting their legs
2	Nervous	The animal startled when humans approach
3	Medium	Cows were calm but they move a lot
4	Calm	Stand calm on the bedding, slash the tail
5	Very calm	Never showed restlessness, fully calm and obedient

3.4 Data preparation and editing

3.4.1 Milking temperament and production data

A total of 3,850 MT records were collected from 2,844 cows in first to third lactation. These cows had 35,379 corresponding test-day records of milk yield and composition. The data were edited to exclude animals without critical information such as birthdate, calving date, parity, days in milk (DIM), milk yield, fat percent or protein percent. Age of cow at calving was restricted to the ranges 18 to 38, 39 to 58 and 59 to 78 months for first, second and third parity, respectively, in order to remove outliers (Mostert *et al.*, 2006; Dube *et al.*, 2008). Test-day milk yields <3.0 kg or >50.4 kg, fat percent <2.66% or >5.56% and protein percent <2.33% or >4.44% were also considered as outliers (Mostert *et al*., 2006) and deleted. Only test-day records falling between 10

and 305 days in milk (DIM) were included in the analyses, in order to factor out colostrum, which lasts 4 to 5 days after calving and contains higher protein and fat soluble vitamins compared to milk (O'Callaghan *et al*., 2021). The standard lactation period is 305 days, after which the cow enters into the dry and resting period in preparation for the next calving and lactation (Kgole, 2013). Lactation stage was defined as early (10-100 DIM), mid (101-200 DIM) or late (201-305 DIM). Age at calving was classified into distinct age groups, as shown in Table 3.2. Herd and test date were concatenated to create herd-test-day (HTD), which was considered as the contemporary group. Two separate data sets were created from the edited data. Data set 1 consisted of 1,348 MT records of 363 cows from four herds, with each cow having 3 or 4 repeated measures (scores). Data set 2 comprised of MT and test-day milk yield and composition records of 2 278 cows, with each cow having a single MT score. Data set 1 was used to compute the repeatability of MT scores and data set 2 was used for the remainder of the analyses.

Table 3.2: Classification of age of cow at calving

Group	Age (months)
1	18-28
2	29-38
3	39-48
4	49-58
5	59-68
6	69-78

3.4.2 Pedigree data

Pedigree records with unknown cow birthdate, dam or sire were deleted. The pedigree file was built around animals in Data set 2 with MT, milk yield, fat and protein percent records, going three generations back. Contemporary groups (HTD) with less than three sires and five records were removed. The final pedigree data set, after editing, consisted of records on 2 278 cows from 16 herds sired by 264 sires, and daughters of 1 309 dams.

3.5 Statistical analysis

3.5.1 Descriptive statistics

Descriptive statistics for MT, milk yield, fat and protein percent were computed using the Proc Means procedure of the Statistical Analysis System (SAS 9.4, 2016).

3.5.1 Repeatability of milking temperament scores

Repeatability of MT scores was calculated from data set 1, by the Varcomp Procedure of the Statistical Analysis System (SAS 9.4, 2016), using the following equation (Caroli, 1998):

$$\textbf{Repeatability} = \frac{\mathbf{Var(Animal)}}{\mathbf{Var(Animal)+Var(Error)}} = \frac{\boldsymbol{\sigma^2_{ANIMAL}}}{\boldsymbol{\sigma^2_{ANIMAL}+\sigma^2_{ERROR}}}$$

Where:

Var(Animal) is the variance of milking temperament scores within animal

Var(Error) is the residual error variance

3.5.2 Environmental factors affecting milking temperament

An analysis of variance was performed on data set 2, using the General Linear Models (GLM) procedure of SAS (SAS 9.4, 2016), to determine non-genetic factors influencing MT. The effects tested were herd-test-day, age of cow at calving, lactation stage and parity. The following model was used for the analysis:

$$y_{ijklm} = \mu + HTD_i + LS_j + P_k + ACC_l + e_{ijklm}$$

Where:

Y_{ijklm} is an observation of milking temperament (1, 2, 3, 4, 5);

μ is the overall population mean;

HTD_i is the fixed effect of the i^{th} herd-test-day;

LS_j is the fixed effect of the j^{th} lactation stage (early, mid, late);

P_k is the fixed effect of the k^{th} parity (1, 2, 3);

ACC_l is fixed effect of the l^{th} age at calving group (1, 2, 3, 4, 5, 6);

e_{ijklm} is the random residual error.

It was assumed that residual errors were independent and identically and normally distributed with mean 0 and variance σ_e^2, i.e.:$e \overset{iid}{\sim} N(0, \boldsymbol{I}\sigma_e^2)$, where σ^2_e is the residual variance and $\boldsymbol{I}$ is an identity matrix. Means for effects that were significant (P<0.05) were separated using the least significant difference (LSD) procedure (α = 0.05).

3.5.3 Estimation of genetic parameters

(Co)variance components among MT, milk yield, protein and fat percent were estimated from data set 2, by linear animal models, using the Restricted Maximum

Likelihood (REML) procedure of the ASReml 4.2 programme (Gilmour *et al.,* 2021). Single trait models were run first, to derive starting values, followed by bivariate analyses to estimate (co)variance components between MT and milk yield, fat and protein percent. The following general models, in matrix notation, were fitted:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix},$$

Where:

y_1 is a vector of observations for milking temperament;

y_2 is a vector of observations for milk yield, fat or protein percent;

b_1 is a vector of fixed effects influencing milking temperament;

b_2 is a vector of fixed effects influencing milk yield, fat or protein percent;

X_1 is an incidence matrix relating fixed effects to milking temperament;

X_2 is an incidence matrix relating fixed effects to milk yield, fat or protein percent;

u_1 is a vector of animal additive genetic effects for milking temperament;

u_2 is a vector of animal additive genetic effects for milk yield, fat or protein percent;

Z_1 is an incidence matrix relating random animal additive genetic effects to milking temperament;

Z_2 is an incidence matrix relating random animal additive genetic effects to milk yield, fat or protein percent;

e_1 is a vector of random residual effects for milking temperament;

e_2 is a vector of random residual effects for milk yield, fat or protein percent.

Fixed effects fitted for milk yield, fat and protein percent were herd-test-day, age of cow at calving, lactation stage and parity (Mostert *et al*., 2006; Kgole, 2013).

Random animal additive genetic effects (u) were assumed to have the distribution *a*~ *N* (0,$A\sigma_u^2$), where A is the additive genetic relationship matrix and σ_u^2 is the animal additive genetic variance. Residual effects (e) were assumed to be distributed with *N* ~ (0,$I\sigma_e^2$), where *I* is an identity matrix, σ_e^2 is the residual variance and *COV* (*u, e*) = *0.*

The (co) variance structure for random effects was assumed to be as follows:

$$\text{var}\begin{bmatrix} u \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_u^2 & 0 \\ 0 & I\sigma_u^2 \end{bmatrix}$$

Heritability was estimated using the following formula (Falconer and Mackay, 1996):

$h^2 = \frac{\sigma^2 u}{\sigma^2 p}$ and $\sigma^2 p = \sigma^2 u + \sigma^2 e$

Where:

h^2 is the heritability;

σ^2_u is the additive genetic variance;

σ^2_p is the phenotypic variance;

σ^2_e is the residual variance.

Genetic and phenotypic correlations for MT with milk yield, fat and protein percent were calculated as follows:

$$r_g = \frac{COV_{gxy}}{\sigma_{gx} + \sigma_{gy}}$$

Where:

r_g is the genetic correlation between traits x and y;

$COV_{g_{xy}}$ is the genetic covariance between traits x and y;

σ_{g_x} is the genetic standard deviation for trait x;

σ_{g_y} is the genetic standard deviation for trait.

$$r_p = \frac{COV_{pxy}}{\sigma_{px} + \sigma_{py}}$$

Where:

r_p is the phenotypic correlation between traits x and y;

$COV_{p_{xy}}$ is the phenotypic covariance between traits x and y;

$\sigma_{p_x}$is the phenotypic standard deviation for trait x;

σ_{p_y} is the phenotypic standard deviation for trait y.

3.5.4 Genetic trend

Estimated breeding values (EBVs) of animals in the pedigree data set, were computed from the co(variance) component estimates by the Best Linear Unbiased Prediction method (Henderson, 1984), using the ASReml 4.2 programme (Gilmour *et al.*, 2021). The genetic trend was then determined by plotting average EBVs by year of birth.

CHAPTER 4: RESULTS

4.1 Introduction

Results from the analyses detailed in Chapter 3 are described in this chapter. These include summary statistics for milking temperament (MT) and milk production traits, the repeatability estimate for MT scores, environmental factors influencing MT, and genetic parameter estimates among MT and milk yield, protein and fat percent. Lastly, the genetic trend for MT is presented.

4.2 Descriptive statistics

Descriptive statistics for MT, milk yield, fat and protein percent are presented in Table 4.1. Milking temperament had a mean score of 3.05±1.32, and the means for daily milk yield, fat and protein percent were 27.22±9.45, 3.84±0.52 and 3.30±0.34, respectively. Coefficient of variation was highest for milking temperament (43.43%), followed by milk yield (34.73%). Fat and protein percent had a much lower coefficient of variation (10.39%).

Table 4.1: Descriptive statistics for milking temperament and milk production traits in South African Holstein cattle.

Traits	Mean	SD	Minimum	Maximum	CV (%)
MT	3.05	1.32	1	5	43.43
Milk (kg/day)	27.22	9.45	3	50.4	34.73
Fat (%)	3.84	0.52	2.66	5.56	13.63
Protein (%)	3.3	0.34	2.33	4.44	10.39

SD=Standard deviation; CV=Coefficient of variation

4.2 Repeatability of milking temperament scores

The repeatability estimate for milking temperament scores was 0.47±0.03.

4.3 Environmental factors influencing milking temperament

Results of the analysis of variance to determine environmental factors influencing milking temperament are summarised in Table 4.2. Milking temperament was significantly influenced by herd-test-day (*P* <0.0001), ACC (*P* <0.0001) and LS (*P* <0.05). Parity, on the other hand, had no significant influence on MT (*P* >0.05).

Table 4.2: Environmental factors influencing milking temperament in South African Holstein cattle.

Factor	Mean Squares	F Value	P-value
HTD	6.54	4.41	<0.0001
ACC	358.85	241.97	<0.0001
LS	6.88	4.64	<0.05
Parity	8.79	4.40	>0.05

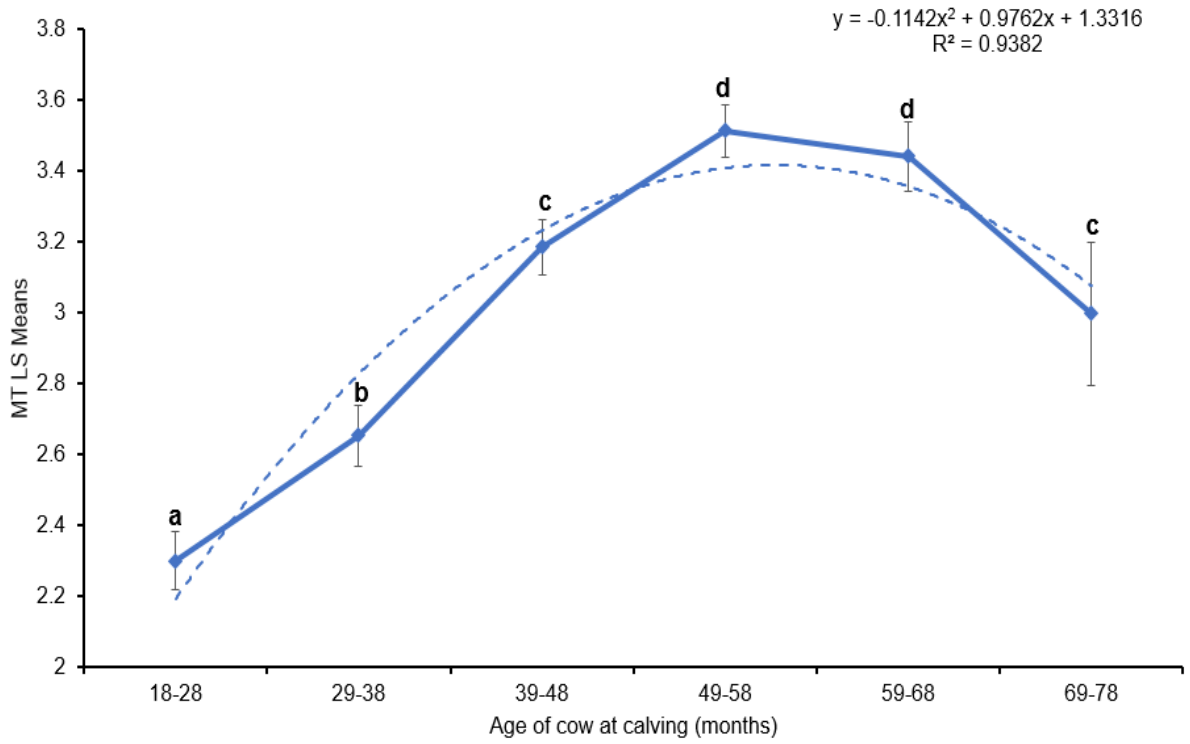
HTD=Herd-test-day; LS=Lactation stage; ACC=Age of cow at calving

4.3.1 Herd-test-day

Herd-test-day had a highly significant effect (*P* <0.0001) on milking temperament, and contributed 42 % of the total variation in milking temperament. Mean scores for HTD groups ranged from 1.13±0.86 to 4.61±1.22.

4.3.2 Age of cow at calving

Figure 4.1 shows the trend in least squares means for milking temperament with age of cow at calving. Milking temperament scores increased with age, peaking at 49-50 months of age, then started to decline with advancing age.

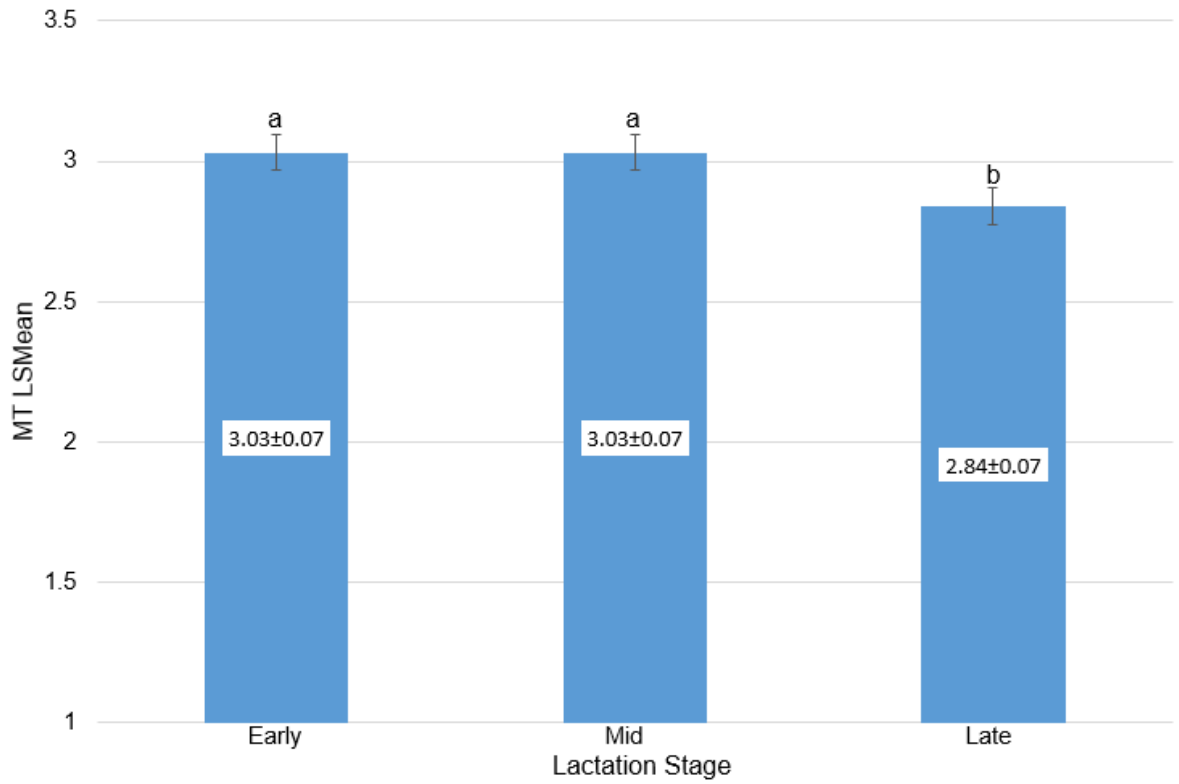


Means with different letters significantly different (P<0.05).

Figure 4.1: Least squares means (LSM) for milking temperament (MT) by age of cow at calving

4.3.3 Stage of lactation

Least squares means for MT scores by stage of lactation are illustrated by the bars in Figure 4.2. Scores were significantly higher (P<0.05) in early and mid than in late lactation. There was, however, no significant difference (P>0.05) in milking temperament between early and mid-lactation. Animals in early and mid-lactation stages had a mean score of 3.03 (medium) while those in late lactation were slightly more nervous (mean = 2.7).



Means with different letters significantly different (P<0.05).

Figure 4.2 : Least squares means for milking temperament (MT) by lactation stage

4.4 Genetic parameter estimates

4.4.1 Heritability

Heritability estimates for MT, milk yield, fat and protein percent are presented in Figure 4.3. The heritability estimate for MT was low (0.05±0.04), while estimates for milk production traits were low to moderate, ranging from 0.11±0.05 for milk yield to 0.24±0.06 for protein percent.

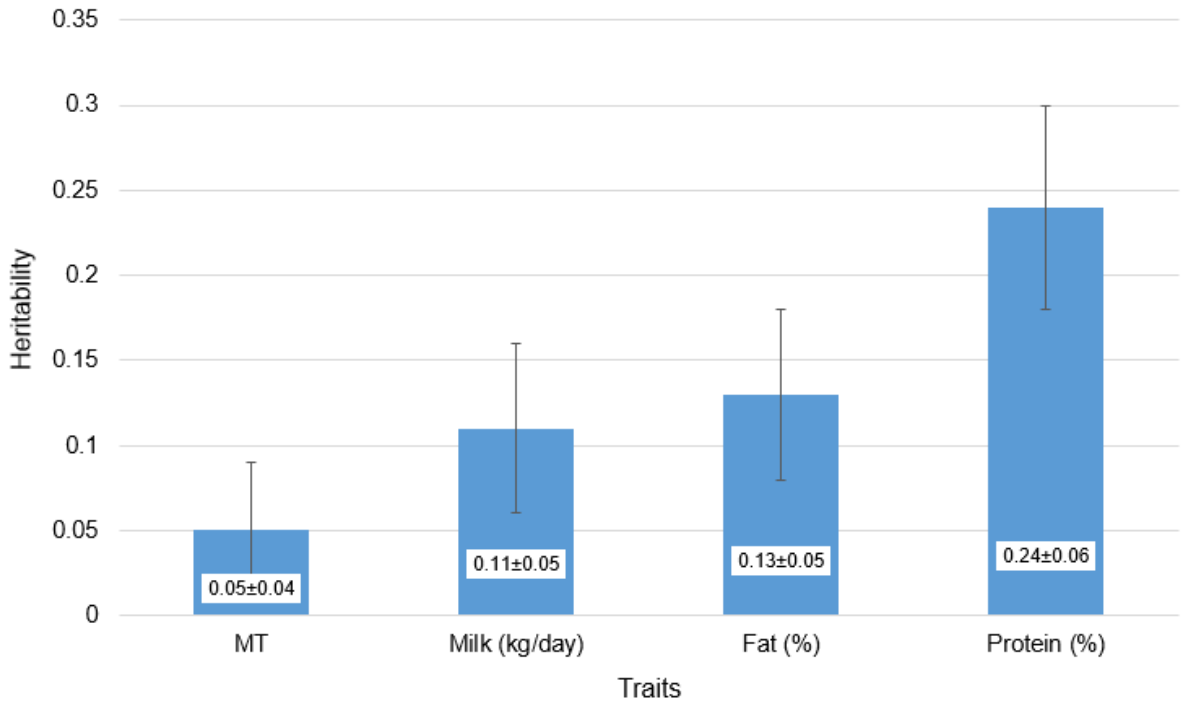


Figure 4.3: Heritability estimates for milking temperament and milk production traits

4.4.2 Genetic and phenotypic correlations

Table 4.3 contains estimates of genetic and phenotypic correlations between MT and milk yield, fat and protein percent. Milking temperament had a moderate and positive genetic correlation of 0.60±0.35 with milk yield. Negative genetic correlations were, however, obtained between MT and fat percent (-0.12±0.24) and protein percent (-

0.30±0.32). Phenotypic correlations were much lower (-0.04±0.02 to 0.25±0.02) compared to the corresponding genetic correlations, although the direction of the relationships remained similar.

Table 4.3: Genetic and phenotypic correlations between milking temperament and milk yield, fat and protein percent in South African Holstein cattle

Trait	r_g ±SE	r_p±SE
Milk (kg/day)	0.60±0.35	0.25±0.02
Fat (%)	-0.12±0.24	-0.06±0.03
Protein (%)	-0.30±0.32	-0.04±0.02

r_g=Genetic correlation; r_p=Phenotypic correlation; SE=Standard error

4.4.3 Genetic trend

Genetic trend for milking temperament, for cows born in the period 2009 to 2019, are presented in Figure 4.4. There was generally no distinct trend, with average estimated breeding values (EBVs) fluctuating from year to year. A peak in average EBVs was observed in 2013, and there was a large dip in 2014-2015. Overall, there was a decrease in mean EBV, at the rate of 0.0009 per year, during the 10-year period. The coefficient of determination for the linear best fit equation was, however, very low (R^2 =12.72%).

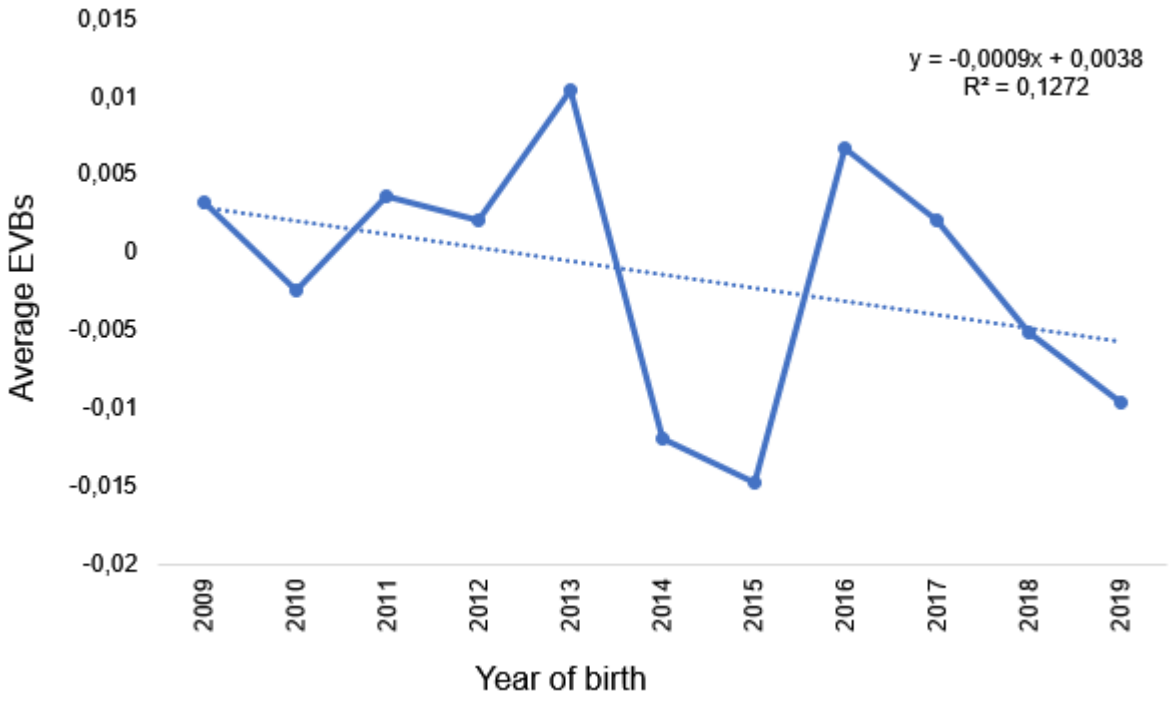


Figure 1.4: Genetic trend for milking temperament (MT) in South African Holstein cattle

CHAPTER 5: DISCUSSION

5.1 Introduction

Milking temperament is an important trait in dairy cattle, as it influences herd profitability, and animal and human welfare. Although it is increasingly being included in selection objectives worldwide, milking temperament has received little attention in South Africa. The current study was primarily aimed at estimating genetic parameters for milking temperament, and its relationships with milk yield, protein and fat percent in South African Holstein cattle. Such parameters are essential for the estimation of breeding values, to enable selection for the trait, in the South African Holstein cattle population. In this chapter, the major findings obtained in the current study and their practical implications are discussed, taking into account existing knowledge from the literature.

5.2 Descriptive statistics

In the current study, cows were evaluated for milking temperament with scores ranging from 1 “very aggressive/nervous” to 5 “very calm”. Milking temperament had a mean score of 3.05±1.32, meaning that, on average, cows were moderately calm. It is, however, difficult to make direct comparison of this mean value for milking temperament with those from other studies, due to disparity among the scoring systems used (e.g. Kruszynski *et al.,* 2013; Antanaitis *et al.*, 2021).

Coefficient of variation for milking temperament (43.43%) was much higher than for production traits, indicating comparatively large variability in MT scores. There are, however, no estimates of coefficient of variation for MT available in the literature to

compare with. Milk production traits had estimates of coefficient of variation ranging from 10.39% for protein percent to 34.73% for milk yield, which were comparable to values obtained recently by Ismael (2021) in Serbian Holstein–Friesian cows.

5.3 Repeatability estimate for milking temperament scores

Repeatability of milking temperament was estimated mainly to assess how closely successive scores on the same cow agree. This depends largely on the consistency of the person doing the assessment, although temporary environmental effects on the animal may also have some influence. Martin and Bateson (1986) proffered that repeatability estimates in the range 0.2 - 0.4 may be regarded as low; those between 0.4 and 0.7 as moderate; and those larger than 0.7 as high. A moderate repeatability estimate (0.47 ± 0.03) was obtained for milking temperament scores recorded in the current study, in close agreement with other studies elsewhere (Kramer *et al.*, 2013; Wethal and Heringstad, 2019; Polupan *et al.*, 2021; Batista-Taborda *et al.*, 2023). The moderate repeatability estimate suggests that repeated scores of milking temperament on a cow are fairly consistent. In addition to indicating consistence of the assessor, this means that it may not be necessary to assess milking temperament repeatedly on a cow, since the first score corresponds reasonably with future scores.

5.4 Non-genetic factors influencing milking temperament

In addition to its use in herd management, knowledge of non-genetic factors affecting a trait is crucial for the accurate estimation of breeding values. This is achieved by fitting them in the models for genetic prediction. In the current study, herd-test-day (HTD), age of cow at calving (ACC) and lactation stage (LS) significantly influenced

milking temperament. These factors were subsequently included in the models for estimating genetic parameters.

5.4.1 Herd-test-day

Herd-test-day, which was regarded as the contemporary group, turned out to be one of the most significant sources of variation in milking temperament (P <0.0001), accounting for 42% of the total variation. This is in agreement with several other studies (Rensing and Ruten, 2005; Szentleleki *et al.,* 2008; Sewalem *et al*., 2011; Haskell *et al*., 2014; Wethal and Heringstad, 2019; Antanaitis *et al*., 2021), and this effect may be attributable to management factors such as animal handling, milking procedure and human approach, which may differ among herds and within the same herd on a day-to-day basis (Costantini *at el.,* 2008; Antanaitis *et al*., 2021; Jaskowski *et al.,* 2023).

5.4.2 Age of cow at calving

Age of the cow at calving came out as another factor with a major influence on milking temperament (P <0.0001), in concurrence several previous studies (Turner *et al*., 2013; Neja *et al*., 2015; Caetano *et al.*, 2017; Cielava *et al*., 2017; Eastham *et al*., 2018; Almasri *et al*., 2020). In the current study, milking temperament scores increased (i.e. cows became calmer) with increase in age of the cow, reaching a peak at 49-68 months of age, and then started to decline (i.e. cows became less calm) with advancing age. This trend could be explained by the fact that young cows are naturally more nervous, and also less familiar with the overall milking procedure, than older cows (Rousing *et al*., 2004; Haskell *et al*., 2012; Marcal-Pedroza *et al*., 2023). It is, however, not clear why cows would become less calm beyond the age of 49-68

months. Surprisingly, though, older Polish Holstein cows were reported to be more aggressive during the milking process compared to younger cows (Kalinska and Slosarz, 2016; Karamfilov, 2022). The reason that older cows were less calm than young cows might be attributable to the fact that older cows become uncomfortable during milking due to the swollen udder from high level of daily milk yield pressure (Szentleleki *et al*., 2015).

5.4.3 Lactation stage

Milking temperament was also significantly influenced by the stage of lactation (P <0.05), with cows in early and mid-lactation being calmer than those in late lactation. This agrees with previous findings by Neja *et al.* (2015) in Polish Holstein-Friesian cows, and may be due to the drop in milk production in late lactation causing cows to be uncomfortable and stressed during the milking process (Chebel *et al*., 2016; Proudfoot *et al*., 2018). Some studies have, however, observed relatively more nervousness/aggressiveness in early-lactation cows compared to those in late lactation (Gergovska *et al.,* 2012; Sawa *et al.*, 2017; Antanaitis *et al.,* 2021). This may partly be caused by the stress of adjusting to new groups, and increased physiological stress, during early lactation.

5.5 Genetic parameter estimates

5.5.1 Heritability estimates

A key objective of this study was to determine the extent to which milking temperament is under additive genetic effects. This is important in assessing the potential for genetic improvement through selection. The (co)variance component estimates derived during

the analysis are also a prerequisite for estimating breeding values for the trait in the population under study. A low heritability estimate for MT (0.05±0.04) was obtained, in agreement with several other studies which reported estimates ranging from 0.03 to 0.14 (Cue *et al.*, 1996; Sewalem *et al.*, 2011; Rinell *et al.*, 2014; Chang and Wang, 2020; Antanaitis *et al.*, 2021; Batista-Taborda *et al.*, 2023). These studies were also based on subjectively assessed MT, and factors such as the scoring scale and analytical models applied seemed to have resulted in a slight variation in the estimates. Larger heritability estimates, ranging between 0.26 and 0.36 were reported for first-parity Danish Holstein cows, based on MT data from objective automatic machine connection evaluation (Stephansen *et al.*, 2018). These estimates suggest that objective assessment of MT using such a method might be better at capturing genetic variance among individual animals, or reducing error variance. The assessment procedure is, however, more time-consuming and can only be used for herds with automatic milking machines. It is evident from the results of the current study, and those from the literature, that MT is under some genetic influence. The low heritability estimate obtained for MT in South African Holstein cattle, in the current study, implies low accuracy of selection; however, this can be improved through approaches such as multiple trait analysis including correlated traits. For example, Eaglen *et al.* (2012) showed that multi-trait analyses including calving ease, stillbirth and gestation length had higher prediction accuracy than univariate analysis for calving ease and stillbirth.

Heritability estimates for milk production traits obtained in the current study ranged from low (0.11±0.05) for milk yield to moderate (0.24±0.06) for protein percent, and were comparable to those from previous studies in South African Holstein cattle (Makgahlela *et al*., 2007; Maiwashe *et al*., 2008; Kgole, 2013; Tlabela, 2020; Van

Niekerk *et al*., 2023) and elsewhere (Peixoto *et al*., 2016; Getahun *et al*., 2020;; Ismael *et al*., 2021; Batista-Taborda *et al.*, 2023; Kinghorn *et al*., 2023). . The relatively higher heritability of these traits may render them useful for improving the accuracy of selection for MT through multiple trait analysis, provided there is considerable genetic correlation.

5.5.2 Genetic and phenotypic correlations

Genetic correlations provide an indication of the expected changes in a trait when selection is applied on other traits. Accuracy of selection for a trait can be improved through multi-trait genetic analysis including traits with which it is genetically correlated. In the current study, milking temperament had a moderate positive association with milk yield, which means that cows with good temperament tended to produce more milk, and *vice versa*. Similar results were also reported for Chinese Holstein cattle by Chang *et al.* (2020), and imply that selection for increased milk yield is likely to result in a correlated improvement in milking temperament. Furthermore, accuracy of selection for milking temperament may be improved through multiple trait analysis including milk yield. Kruszynski *et al.* (2013) also found a positive genetic correlation between milking temperament and milk yield in Brown Swiss cattle, although the relationship was very weak. Contrary to all these studies, Antanaitis *et al.* (2021) observed a negative, although very low, genetic association between milking temperament and milk yield in Lithuanian Holstein cattle.

Genetic correlations between milking temperament and fat and protein percent were low to moderate, and both negative. The negative (undesirable) relationship is plausible, given the fact that fat and protein percent are negatively correlated with milk yield and milking temperament had a positive association with milk yield. Previous

studies elsewhere have, however, reported favourable relationships between milking temperament and fat and protein percent (Kruszynski *et al.*, 2013; Chang *et al.,* 2020; Antanaitis *et al.*, 2021).

Phenotypic correlations between milking temperament and milk production traits followed the same trend as the genetic correlations, but were much lower. The estimate for milk yield was much higher, whereas those for fat and protein percent were comparable, relative to those from a previous study on Lithuanian Holstein cattle (Antanaitis *et al.*, 2021). It, therefore, appears that the relationship between milking temperament and fat and protein percent is weak at both the genetic and phenotypic levels.

5.5.5 Genetic trend for milking temperament

Genetic trend for milking temperament was estimated to determine if there have been any changes in genetic value for the trait in the South African Holstein cattle population in recent years. No consistent trend was observed and, overall, there was a marginal decrease in genetic merit for milking temperament during the 10 year period 2009-2019. This slight decrease could be due to the fact there has been no selection for milking temperament in the South African Holstein cattle population. Similar results were also reported in Polish Holstein-Friesian cattle (Kruszynski *et al*., 2013); however, a minor albeit also irregular increase was observed in Canadian Holstein cattle (Sewalem *et al*., 2011). It is, therefore, imperative to include milking temperament in the breeding objective for South African Holstein cattle, in order to achieve meaningful genetic improvement.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

Herd-test-day, age of cow at calving and lactation stage have a significant influence on milking temperament in South African Holstein cattle and, therefore, should be accounted for in genetic analysis models. Milking temperament scores recorded in the current study were moderately repeatable, indicating reasonable consistency in the assessment process.

A low heritability estimate for milking temperament was obtained for the South African Holstein population, suggesting that low accuracy of selection and genetic progress is achievable. The moderately high genetic correlation obtained between milking temperament and milk yield, however, suggests that there is an opportunity to improve the accuracy of selection through multi-trait analysis including the two traits. There is also a need to implement large-scale recording of milking temperament on South African Holstein herds, as another approach to improve the accuracy of selection.

There has been no discernible change in the genetic merit for milking temperament in the South African Holstein cattle population in recent years. This provides motivation for including milking temperament in the breeding objective, so as to achieve significant genetic improvement. Genetic parameter estimates obtained in the current study may serve to facilitate the estimation of breeding values and, hence, the inclusion of milking temperament in the breeding objective for South African Holstein cattle.

Future work may consider the identification of genomic regions and genes linked with milking temperament. Such information may help to implement marker-assisted selection, and thus accelerate the rate of genetic gain for milking temperament in South African dairy cattle.

REFERENCES

ABDEL-HAMID, S.E., ABDEL-FATTAH, D.M., GHANEM, H.M. AND MANAA, E.A. 2017. Temperament during milking process and its effect on behavioral, productive traits and biochemical parameters in Friesian dairy cows. *Advance Animal of Veterinarian Science*, *5*(12), 508-513.

AGRAVAT, P.H., PATBANDHA, T.K., ODEDRA, M.D., SAVSANI, H.H., AHLAWAT, A.R., KARANGIYA, V.K. AND KALASAVA, S.K. 2023. Effect of Milking Temperament on Milkability Traits and Milk Composition in Gir Cows. *Indian Journal of Veterinary Sciences and Biotechnology*, *19*(1), 87-90.

ALMASRI, O.A.K., ABOU-BAKR, S. AND IBRAHIM, M.A. 2020. Effect of age at first calving and first lactation milk yield on productive life traits of Syrian Shami cows. *Egyptian Journal of Animal Production*, *57*(2), 81-87.

AMIMO, J.O., WAKHUNGU, J.W., INYANGALA, B.O. AND MOSI, R.O. 2007. The effects of non-genetic factors and estimation of genetic and phenotypic parameters and trends for milk yield in Ayrshire cattle in Kenya.

ANTANAITIS, R., JUOZAITIENE, V., JONIKE, V., CUKAUSKAS, V., URBSIENE, D., URBSYS, A., BAUMGARTNER, W., AND PAULAUSKAS, A. 2021. Relationship between Temperament and Stage of Lactation, Productivity and Milk Composition of Dairy Cows. *Animals, 11*(7), 1840.

BALMFORD, A., AMANO, T., BARTLETT, H., CHADWICK, D., COLLINS, A., EDWARDS, D., FIELD, R., GARNSWORTHY, P., GREEN, R., SMITH, P. AND WATERS, H. 2018. The environmental costs and benefts of highyield farming. Nature sustainability, 1(9), 477-485.

BANGA, C.B. AND MAIWASHE, A. 2014, August. Expected Genetic Gain from Newly Developed Selection Indices for South African Holstein Cattle. In *Proceedings of the 10th World Congress of Genetics Applied to Livestock Production, Vancouver, BC, Canada*, 17-22.

BANGA, C.B., NESER, F.W.C. AND GARRICK, D.J. 2014. Breeding objectives for Holstein cattle in South Africa. *South African Journal of Animal Science*, *44*(3), 199-214.

BARRETT, R.T., PETERZ, M., FURNESS, R.W. AND DURINCK, J. 1989. The variability of biometric measurements. *Ringing and Migration*, *10*(1), 13-16.

BATISTA TABORDA, PA., VALENTE, TS., DE LIMA CARVALHAL, MV., DA SILVA, MVGB., PARANHOS, DA. AND COSTA, MJR. 2023. Estimation of genetic parameters for milking temperament in Holstein-Gyr cows. *Frontiers in Animal Science, 4*, 1187273.

BERTENSHAW, C. AND ROWLINSON, P. 2009. Exploring stock managers' perceptions of the human-animal relationship on dairy farms and an association with milk production. *Anthrozoos*, *22*(1), 59-70.

BERRY, D., HARRIS, B., WINKELMAN, A. AND MONTGOMORIE, W. 2005. Phenotypic association between traits other than production and longevity in New Zealand dairy cattle. *Journal of Dairy Science. 88*, 2962-2974.

BOETTCHER, P. 2005. Breeding for improvement of functional traits in dairy cattle. *Italian Journal of Animal Science*, *4*(3), 7-16.

BRANDAO, A.P. AND COOKE, R.F. 2021. Effects of temperament on the reproduction of beef cattle. *Animals*, *11*(11), 3325.

BREUER, K., HEMSWORTH, P.H., BARNETT, J.L., MATTHEWS, L.R. AND COLEMAN, G.J. 2000. Behavioural response to humans and the productivity of commercial dairy cows. *Applied Animal Behaviour Science*, *66*(4), 273-288.

BROTHERSTONE, S., AND GODDARD, M. 2005. Artifcial selection and maintenance of genetic variance in the global dairy cow population. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1459), 1479-1488.

BROWN, E.J. AND VOSLOO, A. 2017. The involvement of the hypothalamopituitary-adrenocortical axis in stress physiology and its significance in the assessment of animal welfare in cattle. *Onderstepoort Journal of Veterinary Research*, *84*(1), 1-9.

BYRNE, T.J., SANTOS, B.F.S., AMER, P.R., MARTIN-COLLADO, D., PRYCE, J.E. AND AXFORD, M. 2016. New breeding objectives and selection indices for the Australian dairy industry. *Journal of Dairy Science*, *99*, 10.

CAETANO, S.L., ROSA, G.J., SAVEGNAGO, R.P., RAMOS, S.B., BERNARDES, P.A., BEZERRA, L.A., LOBO, R.B., DE PAZ, C.C. AND MUNARI, D.P. 2017. Estimation of genetic parameters for longevity considering the cow's age at last calving. *Journal of Applied Genetics*, *58*, 103-109.

CAROLI, A. 1998. Use of the SAS Varcomp Procedure to estimate analytical repeatability. *SAS European Users Group International Proceedings*.

CASSELL, B.G. 2009. Using Heritability for Genetic Improvement [Online]. Available from: https://pubs.ext.vt.edu/404/404-084/404-084.html [29/09/2017].

CHANG, Y., BRITO, F.L., ALVARENGA, A.B AND WANG, Y. 2020. Incorporating temperament traits in dairy cattle breeding programs: challenges and opportunities in the phenomics era. *Animal Frontiers*, *10*, 2.

CHANG, Y., LI, X., ZHANG, H., QI, J., GUO, G., LIU, L. AND WANG, Y. 2019. Genetic analysis for temperament of Chinese Holstein in Beijing area. *Acta Veterinaria et Zootechnica Sinica*, *50*(4), 712-720.

CHANG, Y. AND WANG, Y. 2020. 299 Genetic analysis of temperament and its correlation with production, reproduction, type, health and longevity in Chinese Holstein. *Journal of Animal Science*, *98*(4), 29-30.

CHEBEL, R.C., SILVA, P.R., ENDRES, M.I., BALLOU, M.A. AND LUCHTERHAND, K.L. 2016. Social stressors and their effects on immunity and health of periparturient dairy cows. *Journal of Dairy Science*, *99*(4), 3217-3228.

CHESNAIS, J.P., COOPER, T.A., WIGGANS, G.R., SARGOLZAEI, M., PRYCE, J.E. AND MIGLIOR, F. 2016. Using genomics to enhance selection of novel traits in North American dairy cattle. *Journal of Dairy Science*, *99*(3), 2413-2427.

CIELAVA, L., JONKUS, D. AND PAURA, L. 2017. Lifetime milk productivity and quality in farms with different housing and feeding systems. *Agronomy Research*, *15*(2), 369-375.

CILEK, S. AND SAHIN, E. 2009. Estimation of some genetic parameters (heritability and repeatability) for milk yield in the Anatolian population of Holstein cows. *Archiva Zootechnica*, *12*(1), 57-64.

COCKREM, J.F. 2013. Individual variation in glucocorticoid stress responses in animals. *General and Comparative Endocrinology*, *181*, 45-58.

COOKE, R.F., ARTHINGTON, J.D., ARAUJO, D.B. AND LAMB, G.C. 2009. Effects of acclimation to human interaction on performance, temperament, physiological responses, and pregnancy rates of Brahman-crossbred cows. *Journal of Animal Science*, *87*(12), 4125-4132.

COOKE, R.F. 2014. Bill E. Kunkle Interdisciplinary Beef Symposium: Temperament and acclimation to human handling influence growth, health, and reproductive responses in Bos taurus and Bos indicus cattle. *Journal of Animal Science*, *92*(12), 5325-5333.

COOKE, R.F., LAMB, G.C., VASCONCELOS, J.L.M. AND POHLER, K.G. 2021. Effects of body condition score at initiation of the breeding season on reproductive performance and overall productivity of Bos taurus and B. indicus beef cows. *Animal Reproduction Science*, *232*, 106820.

COOKE, R.F., SCHUBACH, K.M., MARQUES, R.S., PERES, R.F.G., SILVA, L.G.T., CARVALHO, R.S., CIPRIANO, R.S., BOHNERT, D.W., PIRES, A.V. AND VASCONCELOS, J.L.M. 2017. Effects of temperament on physiological, productive, and reproductive responses in Bos indicus beef cows. *Journal of Animal Science*, *95*(1), 1-8.

COSTANTINI, D. 2008. Oxidative stress in ecology and evolution lessons from avian studies. *Ecology letters*, 11, 1238-1251.

COSTILLA, R., KEMPER, K.E., BYRNE, E. M., PORTO-NETO, L. R., ROBERTO CARVALHEIRO, R., PURFIELD, D. C., DOYLE, J. L., BERRY, D. P., STEPHEN S. MOORE, S. S., WRAY, N. R. AND HAYES, B. J. 2020. Genetic control of temperament traits across species: association of autism spectrum disorder risk genes with cattle temperament. *Genetics Selection Evolution, 52*, 51.

CUE, R.I., HARRIS, B.L. AND RENDEL, J.M. 1996. Genetic parameters for traits other than production in purebreds and crossbred New Zealand dairy cattle. *Livestock Production Science*, *45*, 123-135.

CZISZTER, L.T. 2003. Dirijarea Functiei Glandei Mamare. Eurosampa, Timisoara, Romania, 126.

CZISZTER, L.T., GAVOJDIAN, D., NEAMT, R., NECIU, F., KUSZA, S. AND ILIE, D. 2016. Effects of temperament on production and reproductive performances in Simmental dual-purpose cows. *Journal of Veterinarian Behaviour*, *15*, 50-55.

DEPARTMENT OF AGRICULTURE AND RURAL DEVELOPMENT. 2019/2020. South African yearbook.

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES. 2021.

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES. 2012. A Profile of the South African Dairy market value chain.

DODZI, M.S. AND MUCHENJE, V. 2011. Avoidance-related behavioural variables and their relationship to milk yield in pasture-based dairy cows. *Applied Animal Behaviour Science*, *133*(1-2), 11-17.

DUBE, B., DZAMA, K., AND BANGA, C.B. 2008. Genetic analysis of somatic cell score and udder type traits in South African Holstein cows, *South African Journal of Animal Science*, *38*, 1-11.

DUBE, B., DZAMA, K., BANGA, C.B. AND NORRIS, D. 2009. An analysis of the genetic relationship between udder health and udder conformation traits in South African Jersey cows. *Animals*, *3*(4), 494-500.

EAGLEN, S.A., COFFEY, M.P., WOOLLIAMS, J.A. AND WALL, E. 2012. Evaluating alternate models to estimate genetic parameters of calving traits in United Kingdom Holstein-Friesian dairy cattle. *Genetics Selection Evolution*, *44*, 1-13.

EASTHAM, N.T., COATES. A., CRIPPS, P., RICHARDSON, H., SMITH, R. AND OIKONOMOU, G. 2018. Associations between age at first calving and subsequent lactation performance in UK Holstein and Holstein-Friesian dairy cows. *PLoS One*, *13*(6), e0917764.

EGGER-DANNER, C., COLE, J.B., PRYCE, J.E., GENGLER, N., HERINGSTAD, B., BRADLEY, A. AND STOCK, K.F. 2015. Invited review: overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. *Animal*, *9*(2), 191-207.

ERASMUS, L.M. AND VAN MARLE-KOSTER, E. 2021. Moving towards sustainable breeding objectives and cow welfare in dairy production: a South African perspective. *Tropical Animal Health and Production*, *53*(5), 470.

FERNANDEZ-NOVO, A., PEREZ-GARNELO, S.S., VILLAGRA, A., PEREZ-VILLALOBOS, N. AND ASTIZ, S. 2020. The effect of stress on reproduction and reproductive technologies in beef cattle—A review. *Animals*, *10*(11), 2096.

GERGOVSKA, Z., MITEVA, T., ANGELOVA, T., YORDANOVA, D. AND MTEV, J. 2012. Relation of milking temperament and milk yield in Holstein and Brown Swiss cows. *Bulgaria Journal of Agricultural Science, 18*(5), 771-777.

GERGOVSKA, Z.H., MARINOV, I., PENEV, T. AND ANGELOVA, T. 2014. Effect of milking temperament on productive traits and SCC in Black-and-White cows. *International Journal of Current Microbiology and Applied Sciences*, *3*(8), 1-11.

GETAHUN, K., BENEBERU, N. AND LEMMA, Z. 2020. Genetic trend and heritability estimates for milk production traits of Jersey breed in Ethiopian highland environment. *International Journal of Animal Health and Livestock Production Research*, *4*(2), 16-28.

GILMOUR, A. R., GOGEL, B. J., CULLIS, B. R., WELHAM, S. J., & THOMPSON, R. (2021). ASReml User Guide Release 4.2 Functional Specification, VSN International Ltd, Hemel Hempstead, HP2 4TP, UK, www.vsni.co.uk

GYGAX, L., NEUFFER, I., KAUFMANN, C., HAUSER, R. AND WECHSLER, B. 2008. Restlessness behaviour, heart rate and heart-rate variability of dairy cows milked in two types of automatic milking systems and auto-tandem milking parlours. *Applied Animal Behaviour Science*, *109*(2-4), 167-179.

GYGAX, L., NEUFFER, I., KAUFMANN, C., HAUSER, R. AND WECHSLER, B. 2006. Milk cortisol concentration in automatic milking systems compared with auto-tandem milking parlors. *Journal of Dairy Science*, *89*(9), 3447-3454.

HAILE-MARIAM, M., KHANSEFID, M., AXFORD, M., GODDARD, M.E. AND PRYCE, J.E. 2023. Genetic parameters and evaluation of mortality and slaughter rate in Holstein and Jersey cows. *Journal of Dairy Science*, *106*(11), 7880-7892.

HANSEN, L. 2000. Consequences of selection for milk yield from a geneticist's viewpoint. Journal of dairy science, 83(5), 1145-1150.

HARPER, D. G.C. 1994. Some comments on the repeatability of measurements, *15*(2), 84-90.

HASKELL, M.J., GEOFF, S., SIMON, D. AND TURNER. 2014. Genetic selection for temperament traits in dairy and beef cattle. *Frontiers in Genetics*, *5*, 368.

HASKELL, M. J., BELL, D. J., AND GIBBONS, J. M. 2012. Is the response to humans consistent over productive life in dairy cows?, *Animal Welfare*, *21*, 319–324.

HEMSWORTH, P.H. AND COLEMAN, G.J. 2011. Human-animal interactions and animal productivity and welfare. *Human-livestock interactions: The Stockperson and the Productivity and Welfare of Intensively Farmed Animals*, (Ed. 2), 47-83.

HERVE, J., SZENTLELEKI, J. AND TOZSER. 2007. Cattle's behavior-perceptions relationships, studies and measurements of temperaments of temperament. *Animal Welfare Ethology and Housing Systems*, *3*, 27-47.

HINTZ, R.L. AND VAN VLECK, L.D. 1978. Estimation of genetic trends from cow and sire evaluations. *Journal of Dairy Science*, *61*(5), 607-613.

HOLSTEIN. J. 2019, South Africa.co.za.

HULBERT, L.E., CARROLL, J.A., BURDICK, N.C., RANDEL, R.D., BROWN, M.S. AND BALLOU, M.A. 2011. Innate immune responses of temperamental and calm cattle after transportation. *Veterinary Immunology and Immunopathology*, *143*(1-2), 66-74.

INTERBULL. 2022. https://nordicebv.info/wp-content/uploads/2022/08/INTERBULL-breeding-value-August-2022.pdf

INTERGIS. 2000. Integrated registration and genetic information system. Animal production. Agricultural Research Council, South Africa. http://www.arc.agric. za/arc-api/Pages/ARC-API-Homepage.aspx

ISMAEL, H., JANKOVIC, D., STANOJEVIC, D., BOGDANOVIC, V., TRIVUNOVIC, S. AND DJEDOVIC, R. 2021. Estimation of heritability and genetic correlations between milk yield and linear type traits in primiparous Holstein-Friesian cows. *Revista Brasileira de Zootecnia*, *50*.

IMBAYARWO-CHIKOSI, V.E., DUCROCQ, V., BANGA, C.B., HALIMANI, T.E., VAN WYK, J.B., MAIWASHE, A. AND DZAMA, K. 2017. Estimation of genetic parameters for functional longevity in the South African Holstein cattle using a

piecewise Weibull proportional hazards model. *Journal of Animal Breeding and Genetics*, *134*(5), 364-372.

JACOBSEN, J., PALUCCI, V. AND JORJANI, H. 2009. Feasibility of International genetic evaluation for workability traits. *Interbull, bulletin*.

JASKOWSKI, J.M., JASKOWSKI, B.M., HERUDZINSKA, M., TUL, O. AND CIORGA, M. 2023. Contemporary Knowledge on the Assessment of Temperament in Cattle and Its Impact on Production and Reproduction Including Some Immunological, Genetic and Metabolic Parameters. *Animals*, *13*(12), 1944.

KALINSKA, A. AND SLOSARZ, J. 2016. Influence of cow temperament and milking speed on herd life, lifetime milk yield and reasons of cow culling. *Annals of Warsaw University of Life Sciences-SGGW. Animal Science*, *55*.

KARAMFILOV, S. 2022. Study on the temperament of cows of the Aberdeen Angus cattle breed. *Czech Journal of Animal Science*, *67*(1), 8-14.

KASIMANICKAM, R., ASAY, M., SCHROEDER, S., KASIMANICKAM, V., GAY, J.M., KASTELIC, J.P., HALL, J.B. AND WHITTIER, W.D. 2014. Calm temperament improves reproductive performance of beef cows. *Reproduction in Domestic Animals*, *49*(6), 1063-1067.

KASIMANICKAM, V.R., STAKER, C., WILLIAMS, H.M., KASTELIC, J.P. AND KASIMANICKAM, R.K. 2018. Aggressive attempted escape behavior during head-lock restraint reduced reproductive performances in Holstein heifers. *Theriogenology*, *121*, 147-152.

KENTTAMIES, H., NIKKILA, M., MIETTINEN, M. AND ASIKAINEN, J. 2006. Phenotypic and genetic parameters and responses in temperament of silver fox cubs in a selection experiment for confident behaviour. *Agriculture and Food Science, 15*, 340-349.

KGARI, R.D., MULLER, C., DZAMA, K. AND MAKGAHLELA, M.L. 2022. Estimation of Genetic Parameters for Heifer and Cow Fertility Traits Derived from On-Farm AI Service Records of South African Holstein Cattle. *Animals*, *12*(16), 2023.

KGOLE, M.L. 2013. Factors affecting milk urea nitrogen and its relationships with production traits in South African Holstein cattle. MSc Dissertation, University of Pretoria, South Africa.

KINGHORN, B. AND KINGHORN, A. 2023. Management of diversity and inbreeding when importing new stock into an inbred population. *Journal of Heredity*, 27.

KOVACS, L., KEZER, L. AND TOZSER, J. 2013. Measuring Stress Level of Dairy Cows during Milking Using by Geometric Indices of Heart Rate Variability. *Scientific Papers: Animal Science & Biotechnologies/Lucrari Stiintifice: Zootehnie si Biotehnologii*, *46*(1).

KRAMER, M., ERBE, M., BAPST, B., BIEBER, A. AND SIMIANER, H. 2013. Estimation of genetic parameters for novel functional traits in Brown Swiss cattle. *Journal of Dairy Science*, *96*(9), 5954-5964.

KRUSZYNSKI, W., PAWLINA, E., AND SZEWCZUK, M. 2013. Genetic analysis of values, trends and relations between conformation and milk traits in Polish Holstein-Friesian cows. *Archives Animal Breeding*, *56*, 536–546.

KUMAR, A. AND MANDAL, D.K. 2023. Effect of Seasons on Expressions of Milking Parlour Behaviours and Milk Yield of Crossbred Jersey Cows in Tropical Climate. *Asian Journal of Dairy and Food Research*, *42*(1), 60-64.

LACTO DATA. 2021. A Milk SA publication compiled by the Milk Producers' Organization. Vol 24. No1. 05/21

LEVERTON, R. 1989. Wing length changes in individually-marked blackbirds Turdus merula following moult. *Ringing and Migration*. *10*, 17-25.

MADILINDI, M.A., BANGA, C.B. AND ZISHIRI, O.T. 2022. Prediction of dry matter intake and gross feed efficiency using milk production and live weight in first-parity Holstein cows. *Tropical Animal Health and Production*, *54*(5), 278.

MADILINDI, M.A., ZISHIRI, O.T., DUBE, B. AND BANGA, C.B. 2023. Genetic parameter estimates for daily predicted gross feed efficiency and its association with energy-corrected milk in South African Holstein cattle. *Tropical Animal Health and Production*, *55*(5), 339.

MAKGAHLELA, M.L., BANGA, C.B., NORRIS, D., DZAMA, K. AND NG'AMBI, J.W. 2007. Genetic correlations between female fertility and production traits in South African Holstein cattle. *South African Journal of Animal Science*, *37*(3), 180-188.

MAIWASHE, A., NEPHAWE, K.A., VAN DER WESTUIZEN, R.R., MOSTERT, B E. AND THERON, H.E. 2006. Rate of increasing and effective population size in four major South African dairy cattle breeds. *South African. Journal of Animal Science*, *36*, 50-57.

MAIWASHE, A., NEPHAWE, K.A. AND THERON, H.E. 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*(2), 119-125.

MARCAL-PEDROZA, M.G., CAMPOS, M.M., MARTINS, M.F., SILVA, M.V.B., PARANHOS DA COSTA, M.J.R., NEGRAO, J.A. AND SANT'ANNA, A.C. 2023. Is the temperament of crossbred dairy cows related to milk cortisol and oxytocin concentrations, milk yield, and quality? *Plos one*, *18*(6).

MARTIN, P. AND BATESON, P. 1986. Measuring Behaviour: An Introductory Guide. Cambridge: Cambridge University Press.

MAZER, K.A., KNICKERBOCKER, P.L., KUTINA, K.L. AND HUZZEY, J.M. 2020. Changes in behavior and fecal cortisol metabolites when dairy cattle are regrouped in pairs versus individually after calving. *Journal of dairy science*, *103*(5), 4681-4690.

MEKONNEN, M. M., NEALE, C. M., RAY, C., ERICKSON, G. E., AND HOEKSTRA, A. Y. 2019. Water productivity in meat and milk production in the US from 1960 to 2016. Environment international, 132, 105084.

MIGLIOR, F., FLEMING, A., MALCHIODI, F., BRITO, L.F., MARTIN, P. AND BAES, C.F. 2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. *Journal of Dairy Science*, *100*(12), 10251-10271.

MILK PRODUCERS ORGANISATION. 2022. https://mailchi.mp/87a0c9a58849/mpo-bulletin-28-october-2022.

MILKSA. 2021. LactoData.

https://www.mpo.co.za/wpcontent/uploads/2021/05/LACTO-DATA-MAY-21.pdf

MILKSA. 2023. LactoData.

https://milksa.co.za/sites/default/files/2023-06/Lacto%20Data%***20 June***%202023.pdf

MINCU, M., GAVOJDIAN, D., NICOLAE, I., OLTEANU, A.C. AND VLAGIOIU, C. 2021. Effects of milking temperament of dairy cows on production and reproduction efficiency under tied stall housing. *Journal of Veterinary Behavior*, *44*, 12-17.

MORALES-PINEYRUA, J.T., DAMIAN, J.P., BANCHERO, G. AND SANTANNA, A.C. 2022. The effects of heat stress on milk production and the grazing behavior of dairy Holstein cows milked by an automatic milking system. *Journal of Animal Science*, *100*(9), 225.

MOSTERT, B.E., GROENEVELD, E. AND KANFER, F.H.J. 2004. Testday models for production traits in dairy cattle. *South African Journal of Animal Science*, *34*, 35-37.

MOSTERT, B.E., THERON, H.E., KANFER, F.H.J. AND VAN MARLE-KOSTER, E. 2006. Comparison of breeding values and genetic trends for production traits estimated by a lactation model and a fixed regression test-day model. *South African Journal of Animal Science*, *36*(2), 71-78.

MPISANA, Z., JAJA, I.F., OGUTTU, J. AND MUCHENJE, V. 2021. Avoidance behaviour of first lactating cows in a pasture-based dairy system in South Africa. *Journal of Animal Health and Production*, *9*(3), 277-284.

MULLER, R. AND VON KEYSERLINGK, M.A. 2006. Consistency of flight speed and its correlation to productivity and to personality in Bos taurus beef cattle. *Applied Animal Behaviour Science*, *99*(3-4), 193-204.

NATIONAL MILK RECORDING AND IMPROVEMENT SCHEME. 2022. Agricultural ResearchCouncil.https://www.arc.agric.za/arcapi/Pages/Rangelands%20and%20Nutrition/Daiy-Improvement-Scheme.aspx

NEJA, W., SAWA, A., JANKOWSKA, M., BOGUCKI, M. AND KREZEL-CZOPEK, S. 2015. Effect of the temperament of dairy cows on lifetime production efficiency. *Archives Animal Breeding*, *58*(1), 193-197.

NGUYEN, B.T., BRIGGS, K.R. AND NYDAM, D.V. 2023. Dairy production sustainability through a one-health lens. *Journal of the American Veterinary Medical Association*, *261*(1), 12-16.

NYAMUSHAMBA, G.B., TAVIRIMIRWA, B. & BANANA, N.Y.D. 2013. Non-genetic factors affecting milk yield and composition of Holstein-Friesian cows nested

within natural ecological regions of Zimbabwe. *Journal of Animal Science*, *2*, 102-108.

O'CALLAGHAN, T.F., O'DONOVAN, M., MURPHY, J.P., SUGRUE, K., TOBIN, J.T., MCNAMARA, A.E., YIN, X., SUNDARAMOORTHY, G. AND BRENNAN, L. 2021. The bovine colostrum and milk metabolome at the onset of lactation as determined by 1H-NMR. *International Dairy Journal*, *113*, 104881.

ORBAN, M., GALL, K., PAJOR, F., SZENTLELEKI, A., POTI, P., TOZER, J. AND GULYAS, L. 2011. Effect of temperament of Jersey and Holstein Friesian cows on milk production traits and somatic cell count. *Archive Animal Breeding, 54*, 594-599.

PEIXOTO, M.G.C.D., BRUNELI, F.Â.T., BERGMANN, J.A.G., SANTOS, G.D., CARVALHO, M.R.S., BRITO, L.F., PEREIRA, M.C. AND PIRES, M. 2016. Environmental and genetic effects on the temperament variability of Guzerá (Bos indicus) females. *Livestock Research for Rural Development*, *28*(9).

PEZESHKIAN, Z., SHADPARVAR, A.A. AND JOEZY-SHEKALGORABI, S. 2016. Estimation of genetic trends for test-day milk yield by the logarithmic form of wood function using a random regression model. *Iranian Journal of Applied Animal Science*, *6*(1), 43-51.

PROUDFOOT, K.L., WEARY, D.M., LEBLANC, S.J., MAMEDOVA, L.K. AND VON KEYSERLINGK, M.A.G. 2018. Exposure to an unpredictable and competitive social environment affects behavior and health of transition dairy cows. *Journal of Dairy Science*, *101*(10), 9309-9320.

POENA VAN NIEKERK. 2019. Holstein journal. Vol 8(1). December 2019.

POLUPAN, I., SIRIAK, V., STAVETSKA, R., BABENKO, O. AND MOHAMMADABADI, M.R. 2021. Behaviour of cows in the milking parlour and its relationship with milk production and type of NA. *Iranian Journal of Applied Animal Science*, *11*(4), 687-695.

PRIYAM, A., AFONSO, L.O., SCHULTZ, A.G., DINDA, A.K. AND SINGH, P.P. 2023. Hemocompatibility of biogenic phosphorus nano-agromaterials at environmentally relevant and supra-environmental concentrations for occupational exposure. *Environmental Science: Advances*, *2*(2), 313-324.

QUIST, M.A., LEBLANC, S.J., HAND, K.J., LAZENBY, D., MIGLIOR, F. AND KELTON, D.F. 2008. Milking-to-milking variability for milk yield, fat and protein percentage, and somatic cell count. *Journal of Dairy Science*, *91*(9), 3412-3423.

RAMATSOMA, N.I., BANGA, C.B., MACNEIL, M.D. AND MAIWASHE, A. 2014. Evaluation of genetic trends for traits of economic importance in South African Holstein cattle. *South African Journal of Animal Science*, *44*(1), 85-89.

RADU, N.I., LUDOVIC, C.T., SILVIU, S.I., CIPRIAN, M.V., ALEXANDRU, M.E. AND ILIE, D.E. 2023. Effects of Temperament on Milk Related Traits in Simmental Dual-Purpose Cows. *Journal of Animal and Plant Sciences*, *33*(4), 893-902.

RENSING, S., AND RUTEN, W. (2005). Genetic evaluation for milking speed in German Holstein population using different traits in a multiple trait repeatability model. *Interbull bulletin*, (33), 163.

RINELL, E., CARLSTROM, C., HERINGSTAD, B. AND STRANDBERG, E. 2014. Genetic associations between behavior traits recorded by automatic milking systems and temperament of Swedish Holsteins. In *Proc. 10th World Congress on Genetics Applied to Livestock Production, Vancouver, BC, Canada. WCGALP Digital Archive, http://www. wcgalp. Org*, 828.

ROUSING, T., BONDE, M., BADSBERG, J.H. AND SORENSEN, J.T. 2004. Stepping and kicking behaviour during milking in relation to response in human–animal interaction test and clinical health in loose housed dairy cows. Livestock Production Science, 88(1-2), pp.1-8.

SAHEBHONAR, M. 2007. Estimation of genetic trends for production traits and specification of some effective factors on these trends in Iranian Holsteins. *MSc, University of Tehran, Tehran, Iran*.

SANTOS, L.V., K. BRUGEMANN, A. EBINGHAUS, AND S. KONIG. 2018. Genetic parameters for longitudinal behavior and health indicator traits generated in automatic milking systems. *Archives of Animal Breeding*, *61*(2), 161–171.

SANTOS, L., BRUGEMANN, K., SIMIANER, H., AND KONIG, S. 2015. Alternative strategies for genetic analyses of milk flow in dairy cattle. *Journal of Dairy Science*, *98*, 8209–8222.

SAWA, A., BOGUCKI, M., NEJA, W. AND KREZEL-CZOPEK, S. 2017. Effect of temperament on performance of prim parous dairy cows. *Annals of Animal Science, 17*(19), 863-872.

SEWALEM, A., MIGLOIR, F. AND KISTEMAKER, G. 2010. Analysis of the relationship between workability traits and functional longevity in Canadian dairy breeds. *Journal of Diary Science*, *93*(9), 4359-4365.

SEWALEM, A., MIGLOIR, F. AND KISTEMAKER, G. 2011. Genetic parameters of milking temperament and milking speed in Canadian Holsteins. *Journal of Dairy Science*, *94*, 512-516.

SMOLINGER, J. AND SKORJANC, D. 2021. Methods of assessing cattle temperament and factors affecting it: a review. *Agricultura Scientia*, *18*(1-2), 23-37.

SOUTH AFRICAN HOLSTIEN. 2019. *Journal. volume* 8 no1.

SOUTH AFRICAN WEATHER SERVICE. 2021. Annual state of the climate.

SOUTH AFRICAN YEARBOOK. 2019/20. Department of Agriculture, Forestry and Fisheries, South Africa.

STEPHANSEN, R., SFOGH, A. AND NORBERG, E. 2018. Genetic parameters for handling and milking temperament in Danish first-parity Holstein cows. *Journal of Dairy Science, 101*, 11033–11039.

SUTHERLAND, M.A. AND DOWLING, S.K. 2014. The relationship between responsiveness of first-lactation heifers to humans and the behavioral response to milking and milk production measures. *Journal of Veterinary Behavior*, *9*(1), 30-33.

SUTHERLAND, M.A. AND HUDDART, F.J. 2012. The effect of training first-lactation heifers to the milking parlor on the behavioral reactivity to humans and the physiological and behavioral responses to milking and productivity. *Journal of Dairy Science*, *95*(12), 6983-6993.

SZENTLELEKI, A., NAGY, K., SZEPLAKI, K., KEKESI, K. AND TOZSER, J. 2015. Behavioural responses of primiparous and multiparous dairy cows to the milking process over an entire lactation. *Annals of Animal Science*, *15*(1), 185.

SZENTLELEKI, A., HERVE, J., PAJOR, F., FALTA, D. AND TOZER, J. 2008. Temperament of Holstein Friesland cows in milking parlor and its relation with milk production. *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis*, *56*, 201-208.

SZYMIK, B., TOPOLSKI, P. AND JAGUSIAK, W. 2015. Phenotypic characteristics of workability traits in the population of Polish Black- and Red-and-White Holstein-Friesian, Simmental and Polish Red cows (in Polish*). Roczniki Naukowe Zootechniki, 42*, 3–16

SZYMIK, B., TOPOLSKI, P. AND JAGUSIAK, W. 2021 Genetic Parameters of Workability Traits in the Population of Polish Holstein-Friesian Cows Based on Conventional and Genomic Data. *Animals*, *11*, 2443.

TLABELA, M.N. 2020. Heterogeneity of variance for milk production traits between the low and high input dairy production systems of South Africa (Doctoral dissertation).

TSHILATE, T.S., BHEBHE, E., DUBE, B., RHODE, C., MAPHOLI, N.O., MATIKA, O. AND BANGA, C.B. 2021. Genetic parameter estimates for milkability traits and their relationship with somatic cell score in South African Holstein cattle. *Tropical Animal Health and Production*, *53*, 1-8.

TUCKER, J. 2015. Annual milk cattle bulletin, Chemunique, South Africa Msc (Agric) Animal Nutrition. *Pr. Sci. Nat*. 400144/14.

TURNER, B.L, RHOADES, R.D., TEDESCHI, L.O., HANAGRIFF, R.D., McCUISTION, K.C. AND DUNN, B.H. 2013. Analyzing ranch profitability from varying cow sales and heifer replacement rates for beef cow-calf production using system dynamics. *Agricultural Systems*, *114*, 6 -14.

UETAKE, K., KILGOUR, R.J., ISHIWATA, T. and TANAKA, T. 2004. Temperament assessments of lactating cows in three contexts and their applicability as management traits. *Animal Science Journal*, *75*(6), 571-576.

VAN DEN BERGH, J.C. 2010. Externality or sustainability economics?. Ecological Economics, 69(11), 2047-2052.

VAN MARLE-KOSTER, E. AND VISSER, C. 2018. Genetic improvement in South African livestock: can genomics bridge the gap between the developed and developing sectors?. *Frontiers in Genetics*, *9*, 331.

VAN NIEKERK, M., NESER, F., VAN WYK, J. AND DUCROCQ, V. 2023. Comparison of fixed and random regression models for the analysis of milk production traits in South African Holstein dairy cattle under two production systems. *Livestock Science*, *267*, 105125.

WENZEL, C., SCHONREITER-FISCHER, S. AND UNSHELM, J. 2003. Studies on step–kick behavior and stress of cows during milking in an automatic milking system. *Livestock Production Science*, *83*(2-3), 237-246.

WETHAL, K.B. AND HERINGSTAD, B. 2019. Genetic analyses of novel temperament and milkability traits in Norwegian Red cattle based on data from automatic milking systems. *Journal of Dairy Science, 102*, 8221-8233.

SOUTH AFRICA Online (Pty) Ltd. South Africa.co.za. ©2019. The differences between dairy cows South Africa.

YOO, J., SONG, M., PARK, W., OH, S., HAM, J.S., JEONG, S.G. AND KIM, Y. 2019. A comparison of quality characteristics in dairy products made from Jersey and Holstein milk. *Food Science of Animal Resources*, *39*(2), 255.

APPENDIX

ARC • LNR
Excellence in Research and Development

Date: 22nd of December, 2020

Dear Ms T T Siwele,

Re: **"Genetic analysis of milking temperament and its relationship with milk production traits in South African Holstein cattle "**

Your amended application (APAEC 2019/12) for the ethical evaluation of the project entitled **"Genetic analysis of milking temperament and its relationship with milk production traits in South African Holstein cattle"** has been finalized and approved; its reference number is **APAEC [2020/19]**.

I would like to inform you that the project was evaluated and found to be ethically acceptable.

Please note that should any more amendments or changes be made to the protocol, you are obliged to submit an amended application to the Animal Ethics Committee. A hard-copy of this application letter of approval must be available at the site office where animals are kept, including a copy of the protocol, a copy of the signed ethical application, all related SOP's and data monitoring sheets. Further, you will need to inform the committee when the animals will enter the facility, the starting date of the trial and end date of the trial. This approval is valid for two (2) years, projects that are continuing after this will need to re-apply. Failure to comply may lead to withdrawal of the ethical approval. Any neglect finding against this project may result in the applicant being held personally responsible and subjected to the Animal Protection act (Act 71 of 1962).

Regards,

Dr. K-J Leeuw

Chairperson: ARC-API Animal Ethics Committee

Tel. No. 012 672 9320

e-mail: kleeuw@arc.agric.za

Tshwane University of Technology
We empower people

Faculty Committee for Research Ethics-Science [FCRE-SCI]

The FCRE-SCI is a subcommittee of the TUT Senate Committee for Research Ethics.
The TUT Research Ethics Committee is a registered Institutional Review Board (IRB 00005968) with the US Office for Human Research Protections (IORG# 0004997) (Expires 14 January 2023). Also, it has Federal Wide Assurance for the Protection of Human Subjects for International Institutions (FWA 00011501). In South Africa it is registered with the National Health Research Ethics Council (REC-160509-21).

06 September 2021

Ref #: FCRE 2021/05/017 (SCI) (FCPS 03)
Name: Siwele TT
Student/Staff/ID #: 207226149

Siwele TT (207226149)
Animal Sciences
Faculty of Science

FCRE Siwele TT (FA & AREC)

Dear Siwele TT,

Approved & Referred to AREC

Risk Status:	*Low*
Decision:	***FCRE Final Approval Granted. AREC Approval Required.***

Name: Siwele TT
Project title: GENETIC ANALYSIS OF MILKING TEMPERAMENT AND ITS RELATIONSHIP WITH THE MILK PRODUCTION TRAITS IN SOUTH AFRICAN HOLSTEIN CATTLE
Qualification: Masters of Agricultural Science (Animal Science)
Supervisor/s: Prof CB Banga (ARC), Dr B Mtileni, Prof K Nephawe,

Thank you for submitting the **Masters of Agricultural Science** proposal for ethics clearance by the Faculty of Science Committee for Research Ethics (FCRE), Tshwane University of Technology (TUT). We acknowledge the amendments and/or documents submitted. In reviewing the proposal, the comments and notes below are tabled for your consideration, attention and/or notification:

Comments

The changes requested have been made.

…. Important ….

- In view of the Covid-19 pandemic, when applicable, please consider potential changes to:
  (a) The research design and/or research methods, and/or
  (a) The project timeline.
- Contemplate how the impact of the pandemic may be mitigated, particularly for the researcher(s).

*** Very Important: See notes (a) and (b) at the bottom of this letter.

Action

(AREC) - Please await communication from the Animal Research Ethics Committee (AREC).
CONTACT: AREC@tut.ac.za

Tshwane University of Technology

Animal Research Ethics Committee

The TUT Animal Research Ethics Committee is registered in South Africa with the National Health Research Ethics Council (AREC- 010411-009) (Valid until 2021).

July 16, 2021

Ref #: AREC2021/05/003
Name: Siwele TT
Student #: 207226149

Ms TT Siwele
C/o Dr CB Banga
Department of Animal Production
Faculty of Science

Dear Ms Siwele,

Decision: Final approval with comment

Name: Siwele TT
Project title: *Genetic analysis of milking temperament and its relationship with milk production traits in South African Holstein cattle.*
Qualification: Master of Agriculture (Animal Science)
Supervisor: Prof CB Banga
Co-supervisor: Dr BJ Mtileni and Prof K Nephawe

Thank you for submitting the project documents for ethics clearance by the TUT Animal Research Ethics Committee (AREC). In reviewing the documents, the comments and notes below are tabled for your consideration, attention and notification:

Proposal and AREC application form

- The AREC noted and received the clarifications and amendments of the proposal and AREC application form, but is recommending that the changed proposal and clarifications be sent to the chair of the Science Faculty Research Ethics Committee for expedited review and approval. The research team will need informed consent or approval letters from the farms/land owners, not owned by the ARC, that will be involved in this study and the AREC is not mandated to approve human involvement. The AREC approves the animal ethics portion of this study.