ABSTRACT

LOZADA SOTO, EMMANUEL A. Breeding and Management Applications of the Milk Leukocyte Differential. (Under the direction of Dr. Francesco Tiezzi).

Mastitis is by far one of the most economically devastating diseases in dairy cattle. In both its subclinical and clinical form, it is associated with costs related to diagnosis, treatment, production losses, etc. The immune response of the cow is the primary defense against intramammary infections. Associated with the immune response is the recruitment of leukocytes such as neutrophils, macrophages, and lymphocytes that have specific roles for elimination of pathogens and resolution of infection event. The importance of the recruitment of these leukocytes has prompted the development of the milk leukocyte differential (MLD) method to determine the quantity and proportion of the different cells and use this information to diagnose infection events. The primary objective in this dissertation was to examine traits derived from this method for use in everyday mastitis detection and in selection programs for increased mastitis resistance. Our results demonstrated the diagnostic ability of cell thresholds and MLD diagnostic thresholds, significant differences in one or more traits for breed, lactation, day of sample collection, time of sample collection, and quarter position, and the usefulness of MLD traits for use in selection programs highlighted by estimates of heritability and genetic correlations, and results for the relative efficiency of selection against infection using MLD traits as correlated traits.

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Breeding and Management Applications of the Milk Leukocyte Differential

by

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DEDICATION

I dedicate this thesis to my parents Luis R. Lozada-Muñoz and Karen L. Soto-Medina, as well as to my grandparents Arnold Soto-Torres, Imna Medina-Fontaine, and Carmen I. Muñoz-Alvarado.

BIOGRAPHY

Emmanuel André Lozada-Soto was born February 25th, 1995 in San Juan, Puerto Rico to Luis R. Lozada-Muñoz and Karen L. Soto Medina. He attended the University of Puerto Rico at Mayagüez from 2013 to 2017 where he received a Bachelor of Agricultural Science in Animal Science. In Fall 2017, Emmanuel enrolled at North Carolina State University to pursue a Master of Science in Animal Science under the direction of Dr. Francesco Tiezzi.

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CHAPTER 1

Literature Review

INTRODUCTION

Mastitis

Mastitis is a disease that is well known in the dairy industry for its frequency and for the adverse effects on economic return and animal welfare (Jamrozik and Schaeffer, 2012). Mastitis is caused by the entrance of microorganisms into the mammary gland via the teat canal and initiation of an intramammary infection (IMI) (Jashari et al., 2016). Most mastitis cases are classified as either clinical mastitis or subclinical mastitis. Clinical mastitis can vary in severity but it is characterized by observable symptoms such as swelling, edema, and firmness of the infected quarter(s), and may also cause fever, loss of appetite, changes in color of milk, and abnormal secretions from the infected quarter(s), such as clots, flakes, and blood (Divers et al., 2007; Hammer et al., 2012). Apart from the obvious discomfort and loss of welfare of the cow associated with clinical mastitis of any severity, the visibility of affected cows within the herd with this condition makes quick intervention and initiation of management decisions possible. Animals in early lactation and in higher parity lactations are at special risk for contracting clinical mastitis, although first parity animals have higher incidence of clinical mastitis in early lactation than multiparous animals (Compton et al., 2007; Hammer et al., 2012). Subclinical mastitis on the other hand is characterized by a lack of observable symptoms or clinical signs in the milk and udder that can be identified by visual inspection or palpation of the udder, while the mammary gland is inflamed and/or infected and changes in milk composition are occurring (Hammer et al., 2012; Damm et al., 2017; Jaeger et al., 2017). Subclinical mastitis in early lactation has been linked to decreased reproductive performance, which may worsen if infection progresses to clinical stage (Schrick et al., 2001). Some risk factors for subclinical mastitis are geographical location, season, and

term of pregnancy of heifers (Fox, 2009). The costs related with this disease both clinical and subclinical are related to diagnostics, treatment, production losses (loss in milk yield, product quality, discarded milk, and reduction in milk price due to high SCC), labor, culling, and risk of other diseases (Carlén et al., 2004; Halasa et al., 2007). The costs associated with mastitis warrant the development of improved detection of intramammary infections.

Pathogen specific infections

The underlying cause of mastitis, more often than not is bacterial proliferation within the udder, occurring up to 90% of cases in one estimate (Burvenich et al., 1994; Gonçalves et al., 2018). Pathogens responsible for infection have been classified by their pathogenicity as major or minor pathogens, and by their means of proliferation as contagious or environmental pathogens. Contagious pathogens thrive in infected mammary glands and are spread between cows and between mammary quarters of the same cow, while environmental pathogens are present in the environment and infection occurs by contamination of the teats (Oviedo-Boyso et al., 2007). Risk factors for infection with contagious and environmental major pathogens include high herd milk SCC, contact with lactating cows prior to calving, and udder edema for contagious major pathogens, and lack of heifer hygiene and lack of supplementation with vitamins and minerals, for environmental major pathogens (Piepers et al., 2011). The existence of differences among pathogens for changes in milk production (milk loss), quality, and composition related to an IMI has been well established. Gröhn et al. (2004) found differences in milk loss between pathogens, and those responsible for the greatest amount of loss were *S. aureus*, *E. coli*, and *Klebsiella spp.* for first parity animals and

Streptococcus spp., *Staph aureus*, *E. coli*, *Klebsiella spp.*, and *A. pyogenes* for second parity animals. Other studies have found differences between healthy quarters and those infected with contagious and/or gram negative pathogens in daily milk production and total milk loss (Schukken et al., 2009; Bobbo et al., 2017; Gonçalves et al., 2018). Mastitis caused by environmental or contagious major pathogens increases milk SCC values, total protein, total fat, and coagulation time (Sargeant et al., 2001; Bobbo et al., 2017; Gonçalves et al., 2018). *S. aureus* is one of the most commonly isolated contagious major pathogens responsible for subclinical infections due to high prevalence and pathogenicity. The virulence of *S. aureus* is partially due to its use of an extracellular polysaccharide around the cell wall that affects host defense and its mechanism of altering secretory tissue for fibrotic one that affects response to treatment (Baselga et al., 1994; Botaro et al., 2015). Infections with environmental pathogens such as CNS, *S. uberis*, and coliform bacteria are responsible for many cases of mastitis, being prevalent at high levels during calving and decreasing through lactation (Compton et al., 2007; Fox, 2009; Pilla et al., 2012).

Immune response and somatic cells

Mammary gland immunity is a function of the effectiveness of the immune system of a cow. The immune system serves various purposes as it relates to the mammary glands, including prevention of bacterial entrance into the glands, recognition and discrimination between pathogens, elimination of existing infections, and restoration of tissue function (Oviedo-Boyso et al., 2007; Sordillo, 2018). Defense against infection in the bovine mammary gland consists of an anatomical barrier (teat canal and secretions) against pathogens and the immune response (Burvenich et al., 1994; Thompson-Crispi et

al., 2014). Immune response consisting of innate and adaptive immunity plays a key role in the elimination of causal pathogens and quick resolution of an infection event to avoid the immunopathology of mastitis (van den Borne et al., 2011). When exposure to a pathogen occurs the first line of defense for the mammary gland is innate immunity. It is characterized by its rapid activation following exposure and recruitment of somatic cells that have unique functions related to immunity, such as neutrophils (also called PMN), macrophages, lymphocytes, and epithelial cells (Olde Riekerink et al., 2007; Pilla et al., 2012; Sordillo, 2018). The functions of these cells in innate immunity are: phagocytosis (neutrophils and macrophages), intracellular killing of bacteria (neutrophils and macrophages), production of immunoregulatory cytokines and oxylipids (macrophages), removal of debris (macrophages), pathogen recognition (epithelial cells), elimination of infected cells (lymphocytes), and production of antimicrobial proteins (lymphocytes) (Sordillo, 2018). One of the main roles of the innate response is to stimulate the adaptive immune response (Oviedo-Boyso et al., 2007). The adaptive immunity is pathogen specific and regulates or eliminates the signal produced by pathogen recognition, and uses an array of mechanisms to eliminate pathogens (Thompson-Crispi et al., 2014). Macrophages and lymphocytes also have functions in adaptive immunity, involving antigen presentation (macrophages and lymphocytes), production of cytokines (macrophages and lymphocytes) and antigen specific antibodies (lymphocytes) (Sordillo, 2018). The number and distribution of somatic cells has been found to vary between cisternal and alveolar milk (Damm et al., 2017), pathogen specific infections (Leitner et al., 2000), stage of lactation, age of cow, seasons, days, and time of day (Olde Riekerink et al., 2007). In udders free of infection lymphocytes and macrophages are typically the predominant cell types, with higher proportions of macrophages serving as an indicator

of udder health (Leitner et al., 2000; Damm et al., 2017; Sordillo, 2018). In an infected quarter the proportion of neutrophils increases dramatically, forming the first line of defense, in fact the effectiveness of intramammary defense is dependent on this increase (Paape et al., 2003). Leitner et al. (2000) found that neutrophils were the main cell type in udders infected with acute *E. coli* and *S. aureus*. Overall, studying the immune response is a way to understand the inflammatory processes that are occurring, helping us identify which cows are undergoing an infection event and expediting treatment.

MASTITIS DETECTION METHODS

Many methods have been developed in recent decades to detect and diagnose an intramammary infection. Among the most popular and readily available are the California Mastitis Test (CMT), Somatic Cell Count (SCC), and bacteriological culturing. Recently a new tool has been developed, called Milk Leukocyte Differential (MLD), which quantifies somatic cells by type, opening up new avenues into mastitis monitoring and mastitis resistant research.

California Mastitis Test

The CMT is a qualitative measurement of the somatic cell count in the milk that has been used in fresh cow udder health monitoring as a screening test for subclinical mastitis (Dingwell et al., 2003; Anderson et al., 2010). It consists of adding a reagent to a quarter milk sample that disrupts the walls of somatic cells in the milk causing a thickening reaction that is scored indicating severity of infection (Leach et al., 2008). The subjective nature of the CMT scoring makes this method lack in accuracy. Dingwell et al. (2003) found that CMT has less than ideal sensitivity and specificity, which limits

effectiveness of use in individuals but might serve to develop herd level udder health profiles. Sargeant et al. (2001) found that increasing CMT threshold for infection, decreased the sensitivity and increased the specificity for identifying infected quarters. Anderson et al. (2010) found that CMT was not associated with significant mean differences in production which indicates limited use of CMT to predict milk loss associated with an IMI.

Somatic Cell Count

Somatic cell count has become the premier tool for mastitis monitoring and detection worldwide (Damm et al., 2017). SCC is a measurement of the total number of somatic cells in milk (cells/mL). It is used to evaluate udder health (presence and severity of infection and/or inflammation) and milk quality, where increased SCC indicates increased infection and/or inflammation and worsening milk quality (Leitner et al., 2000; Hand et al., 2012). The most widely used SCC threshold to distinguish between infected and uninfected quarters is a threshold of 100,000 cells/mL (Schwarz et al., 2010, 2011; Bobbo et al., 2017), though other thresholds have been identified and studied. For example, Jaeger et al. (2017) conducted a study where the optimal threshold for IMI detection was 150,000 cells/ml, meanwhile Jashari et al. (2016) stated that threshold values for detection of subclinical mastitis should be tailored to pathogen groups of interests. Increase in SCC threshold has been found to decrease the sensitivity and increase the specificity of identifying infected quarters (Sargeant et al., 2001) , which indicates that a preference of minimizing false positives over false negatives and vice versa might be a factor to consider when selecting an appropriate SCC threshold. Significant differences in SCC have been found between different times of day,

specifically between milkings, and vary between quarters (Olde Riekerink et al., 2007). Research into the relationship between SCC and cow production traits such as milk yield and milk components has been abundant. Anderson et al. (2010) found that for SCC in 4 categories in respect to number of infected quarters (0, 1, 2, 3 or 4), SCC thresholds of 200,000 cells/mL, 300,000 cells/mL, and 400,000 cells/mL were significantly associated with differences in summit milk, and the SCC threshold of 400,000 cells/mL was significantly associated with differences in 150d milk production. Hand et al. (2012) found number of test days with increased SCC to be a major risk factor for milk loss. With respect to the effect of SCC on milk composition, Malek dos Reis et al. (2013) found that increased SCC negatively affected the lactose and nonfat solids content of milk, while at the same time having the opposite effect on protein, total solids content, and fat yield. Similar results for effect of SCC on fat yield were found by Botaro et al. (2015). Milk loss because of high SCC has been linked to the parity of the animal and stage of lactation. Multiparous cows have increased milk loss as compared to their primiparous counterparts when SCC increases and have a higher risk of developing clinical mastitis than primiparous cows when both groups have low composite SCC (Carlén et al., 2004; Hagnestam-Nielsen et al., 2009; van den Borne et al., 2011). Stage of lactation has been found to be associated with production losses related to increased SCC, with animals in the latter stages of lactation suffering from the biggest production losses related to milk yield, with a possible explanation being lessening of compensatory ability of uninfected quarters as lactation progresses (Hagnestam-Nielsen et al., 2009). In summary, SCC is an invaluable tool for identifying quarters that are inflamed either by infection or other reasons. It has been reported to aid in fresh cow monitoring and

identification of animals with subclinical mastitis and animals at risk for clinical mastitis, possibly indicating level of production in later stages of lactation (Anderson et al., 2010).

Bacteriological Culturing

Bacteriological culturing is the standard method to diagnose intramammary infections. It is the most reliable method and the only one that elucidates the microbiological etiology of mastitis (Sargeant et al., 2001; Anderson et al., 2010). Since defining infection status is one of the biggest challenges in mastitis research, triplicate sampling for bacteriological culturing has been used as the gold standard to which the accuracy of other methods is measured against (Piepers et al., 2011). Since mastitis is caused by many pathogens that differ in pathogenesis, epidemiology, and symptoms caused, bacteriological culturing is used to identify the pathogens responsible so appropriate management decisions are implemented (proper antibiotic treatment) (Gröhn et al., 2004; Jaeger et al., 2017). The limitations of bacteriological culturing include high cost of sampling compared to other methods and the logistics involved if triplicate sampling of all quarters at calving for development of a herd pathogen profile is desired (Sargeant et al., 2001; Anderson et al., 2010). Nevertheless, if used in combination with other methods that accurately identify quarters in need of culturing, it is highly useful for mastitis monitoring.

Milk Leukocyte Differential

The MLD, which is used interchangeably with differential somatic cell count (DSCC), is a relatively new method of mastitis detection. It has only been used recently for dairy cattle mastitis monitoring. It consists of analysis of a milk sample where the

quantity of the different leukocytes is determined and expressed as cells per mL of milk. MLD analysis is focused on determining the number of neutrophils, macrophages, and lymphocytes, and their proportions. The two main methods for obtaining cell differentials are microscopy and flow cytometry. Microscopy was the preferred method in the past for being simple and cost effective, but it suffers from not being especially fast and having wide variation in results and poor repeatability due to differences in materials for sampling, differences in preparation, and differences between technicians (Redelman et al., 1988). Flow cytometry on the other hand has higher accuracy and is faster to perform (Pilla et al., 2013). Use of cell differentiation can reveal underlying inflammatory processes in mammary glands that have SCC levels below the usual threshold of $100x10^3$ cells/mL (Schwarz et al., 2010, 2011; Pilla et al., 2013). The use of cell differential methods extends to early lactation cow monitoring, where determination of the leukocyte proportions can help us assess udder health in this critical period and give us indications of future lactation production measures (Anderson et al., 2010). Anderson et al. (2010) found that for day 3 post calving, milk neutrophil percentage thresholds of 35% and 40% were associated with significant differences in 150-day milk when considered in 2 categories (cows with no quarter above threshold and cows with at least 1 quarter above threshold), neutrophil percentage threshold of 30% was associated with significant differences for summit milk when considered in 4 categories (in respect to amount of quarters above threshold), and total absolute neutrophil thresholds of $150x10^3$ cells/mL, $200x10^3$ cells/mL, and $250x10^3$ cells/mL were significantly associated with summit milk when considered in 2 categories, and with both 150-day milk and summit milk when considered in 4 categories. One drawback of cell differentiation methods is that they were primarily developed for analysis of individual cow milk samples which precludes the

development of composite and herd average parameters (Damm et al., 2017). Nevertheless, cell differential methods are useful in helping detect initial phases of inflammation, define the stage of infection, select quarters for further analysis with bacteriological culturing, determine the success of antibiotic treatments, and monitor recovery among other things (Redelman et al., 1988; Koess and Hamann, 2008).

GENETIC SELECTION FOR MASTITIS RESISTANCE

Mastitis resistance is a highly complex trait involving genetic, physiological, and environmental factors (Rupp and Boichard, 2003). At its core resistance to any malady implies the ability to avoid factors causing disease. Understanding the underlying genetic architecture of resistance has been a driver of research in this area. Selection for resistance is possible due to the existence of variability related to additive genetic effect of mastitis resistance, even though the consensus is that it makes up a small part of total phenotypic variance (Rupp and Boichard, 2003; Neuenschwander et al., 2012). Phenotype measures to evaluate and improve mastitis resistance include direct methods (infection diagnosis by bacteriological culturing), indirect methods (such as SCC, udder conformation, and milking speed), and combinations of both (Rupp and Boichard, 2003; Bloemhof et al., 2009; Thompson-Crispi et al., 2014).

Direct Selection

Genetic progress using direct selection against infection requires extremely accurate recording of health events during an animals life and repeatability of the data recorded, which has not been the case in the United States and most of the world, except in Scandinavia where direct selection for resistance has been taking place successfully for

decades (Neuenschwander et al., 2012; Parker Gaddis et al., 2014). When studied, estimates of the heritability of mastitis tend to be low. Examples of published estimates for unspecified, clinical and subclinical mastitis are presented in table 1. Overall, unspecified mastitis, clinical mastitis, and subclinical mastitis have similar estimates of heritability in the literature ranging from 0.02 to 0.09, 0.02 to 0.07, and 0.03 to 0.04 respectively. Mastitis incidence from animals of different parity can be thought of as distinct traits, with some published estimates of heritability also appearing in table 1. In the literature, first parity animals had heritability estimates ranging from 0.03 to 0.08 for clinical mastitis, of 0.17 for subclinical mastitis, and from 0.06 to 0.10 for unspecified mastitis, while animals with more than one parity had estimates ranging from 0.03 to 0.10 for clinical mastitis, 0.07 to 0.08 for subclinical mastitis, and from 0.05 to 0.06 for unspecified mastitis. Genetic correlation between mastitis incidence at different parities seems to increase with proximity of the lactations. Heringstad et al. (2005) estimated genetic correlations of 0.73, 0.67, and 0.73 between clinical mastitis at 1st parity and clinical mastitis at 2nd parity, clinical mastitis at 1st parity and clinical mastitis at 3rd parity, and clinical mastitis at 2nd parity and clinical mastitis at 3rd parity respectively. Similarly Bloemhof et al. (2009) estimated genetic correlations of 0.88, 0.63, and 0.91 between clinical mastitis at 1st parity and clinical mastitis at 2nd parity, clinical mastitis at 1st parity and clinical mastitis at 3rd parity, and clinical mastitis at 2nd parity and clinical mastitis at 3rd parity respectively. Even though direct selection for resistance is feasible and has been done, it is hindered by low heritability of mastitis, inaccurate health recording, higher cost of phenotyping, and potential inadvertent increase in susceptibility to other diseases (Thompson-Crispi et al., 2012).

Indirect Selection

Indirect selection for mastitis resistance is more commonly done and can use genetically correlated traits related to inflammation, immune response, other diseases, conformation, and production measures (Jamrozik and Schaeffer, 2012). To improve disease resistance, (Thompson-Crispi et al., 2012) studied traits related to immune response, including cell mediated and antibody mediated response. These measures were found to have low to medium heritability and positive correlation with reproductive and conformational traits. Indirect selection can also be performed while trying to increase resistance to other diseases, such as ketosis, lameness, cystic ovaries, displaced abomasum, retained placenta. Parker Gaddis et al. (2014) estimated that mastitis has low to moderate and positive genetic correlations with cystic ovaries, displaced abomasum, ketosis, lameness, and retained placenta in first parity animals in both pedigree and genomic based analysis, while having low to moderate positive genetic correlations with cystic ovaries, displaced abomasum, and retained placenta in later parity animals in both pedigree and genomic based analysis. A similar study done by Zwald et al. (2004) found low positive genetic correlations between mastitis and displaced abomasum, ketosis, lameness, and cystic ovaries. Both studies found that mastitis and metritis share a low negative genetic correlation, which implies that selection for resistance to either trait might lead to susceptibility to the other. Selection for reproductive and production traits can also have an impact in mastitis phenotype. Parker Gaddis et al. (2014) estimated low and negative genetic correlations between daughter pregnancy rate, productive life, and net merit, and mastitis, as well as positive correlations with milk yield and somatic cell score. The genetic relationship between conformation traits in dairy cattle and mastitis has also been heavily studied. The consensus is that mastitis has a low to moderate and

negative genetic correlation with traits such as udder depth, udder balance, fore udder attachment, and body conditioning score (Lassen et al., 2003; Govignon-Gion et al., 2012; Koeck et al., 2012b). Govignon-Gion et al. (2012) found the following genetic correlations between clinical mastitis and conformation traits: udder depth (ranges from -0.54 to -0.30 depending on breed), udder balance (ranges from -0.18 to -0.15 depending on breed), fore udder attachment (-0.13 for Holsteins), and body conditioning score (-0.32 for Holsteins). Koeck et al. (2012b) found similar estimates for udder depth (-0.36) and fore udder attachment (-0.24). Results like these indicate that selection for improved conformation would be beneficial for decreasing mastitis incidence. Nevertheless, the most important correlated traits used in dairy producing countries are somatic cell count traits. Lactation average somatic cell count (LASCC), lactation average somatic cell score (LASCS), standard deviation of somatic cell count (SCC SD), standard deviation of somatic cell score (SCS SD), and number of test day records above a certain threshold (TD) are some of the traits that have been used as indicators of mastitis for selection. A few of the published estimates for the heritability of somatic cell count traits can be found in table 2. Genetic correlations of somatic cell count traits with mastitis were similar between studies, with correlations with clinical mastitis typically ranging from 0.50 to 0.83 and with subclinical mastitis ranging from 0.67 to 0.98. No studies involving estimation of genetic parameters for traits derived from cell differentiation methods for genetic evaluation of mastitis were found. Therefore, further research in this area is needed to determine usefulness of such traits. Overall, indirect selection for mastitis resistance using genetically correlated traits has been proven effective due to lower cost of phenotyping, availability of records, higher heritability, and high genetic correlations with infection.

Table 1. Heritability estimates for mastitis traits.

Trait	Breed	Country	Heritability	Reference
Unspecified mastitis	Holstein	Canada	0.02	(Koeck et al., 2012a)
Unspecified mastitis	Not specified	USA	0.09	(Zwald et al., 2004)
Clinical mastitis	Holstein	Canada	0.05	(Neuenschwander et al., 2012)
Clinical mastitis	Not specified	Netherlands	0.03 (dataset A) and 0.02 (dataset B)	(de Haas et al., 2008)
Subclinical mastitis	Not specified	Netherlands	0.04 (dataset A) and 0.03 (dataset B)	(de Haas et al., 2008)
Clinical mastitis	Montbeliarde Normande Holstein	France	0.02 0.02 0.02	(Govignon-Gion et al., 2012)
Clinical mastitis	Norwegian Red	Norway	0.07	(Holtsmark et al., 2008)
Unspecified mastitis	Not specified	Canada	0.07	(Koeck et al., 2012b)
Unspecified mastitis	Holstein	Denmark	0.04	(Lassen et al., 2003)
Clinical mastitis	Swedish Red and Swedish Holstein	Sweden	0.04 (weekly records) 0.04 (monthly records)	(Urioste et al., 2010)
Clinical mastitis	Swedish Holstein	Sweden	0.08 (parity 1) 0.08 (parity 2) 0.07 (parity 3)	(Urioste et al., 2012)
Subclinical mastitis	Swedish Holstein	Sweden	0.17 (parity 1) 0.16 (parity 2) 0.13 (parity 3)	(Urioste et al., 2012)
Clinical mastitis	Holstein	Czech Republic	0.10 (parity 1) 0.10 (parity 2) 0.09 (parity 3)	(Zavadilová et al., 2015)
Clinical mastitis	Norwegian Red	Norway	0.08 (parity 1) 0.07 (parity 2) 0.07 (parity 3)	(Heringstad et al., 2005)
Clinical mastitis	Holstein, Dutch-Friesian, and Meuse-Rhine-Yssel	Netherlands	0.03 (parity 1) 0.03 (parity 2) 0.04 (parity 3)	(Bloemhof et al., 2009)
Unspecified mastitis	Not specified	USA	0.06 (first parity, pedigree analysis) 0.10 (first parity, genomic analysis) 0.06 (later parities, pedigree analysis) 0.05 (later parities, genomic analysis)	(Parker Gaddis et al., 2014)

Table 2. Heritability estimates for somatic cell count traits.

Trait[1]	Breed	Country	Heritability	Reference
LASCC (5-170)	Danish Holsteins	Denmark	0.20	(Sørensen et al., 2009)
LASCC (5-300)	Danish Holsteins	Denmark	0.23	(Sørensen et al., 2009)
LASCS	Holstein	Sweden	0.14 (parity 1) 0.13 (parity 2) 0.10 (parity 3)	(Carlén et al., 2004)
LASCS (0-150)	Holstein	Canada	0.11	(Koeck et al., 2012c)
LASCS (151-250)	Holstein	Canada	0.11	(Koeck et al., 2012c)
LASCS	Not specified	Netherlands	0.08 to 0.13	(de Haas et al., 2008)
LASCS	Norwegian Red	Norway	0.12	(Holtsmark et al., 2008)
LASCS (0-150)	Not specified	Canada	0.10	(Koeck et al., 2012b)
SCS SD	Not specified	Canada	0.04	(Koeck et al., 2012b)
TD (>500)	Not specified	Canada	0.06	(Koeck et al., 2012b)
TD (41-80)	Swedish Red and Swedish Holstein	Sweden	0.15 (weekly records) 0.08 (monthly records)	(Urioste et al., 2010)
TD (151-500)	Swedish Red and Swedish Holstein	Sweden	0.16 (weekly records) 0.22 (monthly records)	(Urioste et al., 2010)
TD (>500)	Swedish Red and Swedish Holstein	Sweden	0.16 (weekly records) 0.22 (monthly records)	(Urioste et al., 2010)
SCC SD	Swedish Red and Swedish Holstein	Sweden	0.10 (weekly records) 0.13 (monthly records)	(Urioste et al., 2010)

[1]LASCC = lactation average somatic cell count (days averaged in parenthesis); LASCS= lactation average somatic cell score (days averaged in parenthesis); SCC SD = Somatic cell count standard deviation; SCS SD = Somatic cell score standard deviation; TD (41-80, 151-500, >500) = number of test days with SCC above threshold (in parenthesis).

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CHAPTER 2

Analysis of milk leukocyte differential traits for use in management and breeding for reduced mastitis incidence.

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ABSTRACT

The aim of this study was to assess the viability of traits derived from milk leukocyte differential (MLD) for inclusion in selection programs focused on decreased mastitis susceptibility and for use in fresh cow mastitis monitoring. Quarter milk samples were collected from Holstein and Jersey cows on day 4 and 11 post calving. Samples were analyzed using MLD where cell counts for total leukocytes, neutrophils, macrophages, and lymphocytes were obtained, as well as a quarter infection diagnosis. Traits derived from MLD included cell scores (total leukocyte score (TLS), neutrophil score (NS), macrophage score (MS), and lymphocyte score (LS)), cell proportions (neutrophil percentage (NP), macrophage percentage (MP), and lymphocyte percentage (LP)), cell thresholds (total leukocyte count threshold (tTLC), neutrophil threshold (tN), macrophage threshold (tM), and lymphocyte threshold (tL)), and MLD diagnostic thresholds (diagnosis at threshold setting A (diagA), threshold seting B (diagB), and threshold setting C (diagC)). Microbiological culturing was conducted to determine infection status to compare our MLD diagnosis, serve as an indicator of infection, and to determine pathogen occurrence. Traits derived from the microbiological analysis included occurrence of major pathogens (MaP), occurrence of minor pathogens (MiP), and infection (INF). Data analysis was done using two linear mixed models, the first one (Model I) was used on all traits for the estimation of the fixed effects of breed, lactation number, day of sample collection, time of sampling and quarter location, and the random effects of animal and week of sampling. The second model (Model II) was used for the estimation of variance components and genetic parameters, and included the fixed effects in Model I, and the random effects of additive genetic effect and permanent environmental effect. Results show all fixed effects were significant for one or more of

the analyzed traits. Heritability estimates include low to moderate heritability estimates for cell score and cell percentage traits, and moderate to high estimates for cell threshold, MLD diagnosis, and infection traits. Phenotypic and genetic correlations among and between cell score and cell percentage traits were as expected due to their shared genetic determination related to immune response. Phenotypic and genetic correlations between all traits and infection and calculated relative efficiency of selection for infection using the correlated traits showed that most traits analyzed in this study have value for inclusion in decreased mastitis susceptibility breeding programs, specially MLD diagnostic traits.

Key words: mastitis susceptibility, somatic cells, milk leukocyte differential, genetic parameters

INTRODUCTION

Mastitis is well known as a cause of economic loss, due to reduced production, diagnostic and treatment costs, and premature culling of cows (Halasa et al., 2007). This includes both clinical mastitis (CM) as well as subclinical mastitis (SM). Mastitis has profound effects on many aspects of dairy production; having been associated with decreased yields and changes in milk quality and composition (Bobbo et al., 2017). SM is especially concerning because it oftentimes remains undetected due to the lack of external symptoms; meanwhile, the underlying infection and its effects persist. Somatic cell count (SCC) is a measurement that has been adopted as one of the most widely used monitoring tools for mastitis (Damm et al., 2017). SCC measures the total number of somatic cells in milk (cells/mL) and is used to evaluate udder health, milk quality, and severity of infection, with cell count levels at or above 100×10^3 cells/mL generally indicating inflammation (Schwarz et al., 2011; Hand et al., 2012; Bobbo et al., 2017).

However, quantification of the different cell types and their proportions is not possible using SCC alone. For this purpose, the milk leukocyte differential (MLD) has been developed. MLD is a tool that separates and quantifies the somatic cells in the milk responsible for the immune response in the mammary gland. It is useful in monitoring changes in cell proportions, which can be used to detect inflammation in the early stages of infection before SCC levels become alarming (Pilla et al., 2012; 2013).

Progress due to genetic selection towards decreased susceptibility to mastitis has not advanced at a rate equal to yield traits. Current research has focused on exploring the genetic relationships between mastitis and SCC. Mastitis and SCC traits have been found to share low heritability estimates and moderate positive genetic correlations between them (Koeck et al., 2012). This relationship has resulted in the routine inclusion of predictor traits such as SCC in mastitis resistance breeding programs (Martin et al., 2018). However, similar research with MLD traits to determine their merit and possible advantage over SCC traits as tools for both diagnosis and selection has been so far scarce. Therefore, the objective of this study was to determine the variance components and genetic parameters of indicator traits derived from MLD and of intramammary infection (INF) diagnosed via bacteriological culturing, and to examine the feasibility of inclusion of MLD traits in decreased mastitis susceptibility breeding programs. Additionally, we sought to evaluate the use of MLD as a tool for subclinical mastitis screening in early lactation.

MATERIALS AND METHODS

Animals

Animal use was approved by the North Carolina State University Institutional Animal Care and Use Committee. Quarter milk samples were collected from enrolled recently calved dairy cows on both days 4 and 11 post-calving (calving date treated as day 0) from December 2016 to November 2017. The study included a total of 127 animals from the Holstein (HO, n=82) and Jersey (JE, n=45) breeds ranging from lactation 1 to 6. Cows were housed at the Dairy Education Unit at North Carolina State University (NCSU). The herd had an average milk yield of 11,762 kg/yr and an average bulk tank SCC of 177,000 cells/mL during the study. Animals were fed a TMR of corn silage, sorghum silage, grass hay, soy hulls, citrus pulp, and grain mix. Dry cows had free access to pastures or free stalls at their discretion, while the milking herd had limited access to pastures and were milked twice a day at a 12-hour interval in a parallel milking parlor. Cows treated with antibiotics from the period of a month before calving to day 4 post calving or treated between day 4 and 11 post calving were not eligible for the study.

Sample Collection and Analysis

Quarter milk samples (N=987) were collected either during the morning or evening milking in quarter-based sampling chambers (Q4, Advanced Animal Diagnostics, Inc., Morrisville, NC) for determination of MLD and aseptically collected quarter milk samples (N=973) in 13mL vials for microbiological culturing. Prior to collection, quarters were aseptically prepared using steps outlined in the Laboratory and Field Handbook on Bovine Mastitis (National Mastitis Council (U.S.), 1987). Samples for microbiological culturing were collected from the foremilk after expression of 2-3

streams of milk, after which the samples for MLD analysis were collected. Samples were then transported to the NCSU Veterinary College, where samples underwent MLD analysis and duplicate samples were frozen at -20°C for subsequent microbiological analysis.

Microbiological Analysis

In total 973 sterile milk samples were collected from both day 4 (N=511) and day 11(N=462) post calving. A total of 95 samples were found to be contaminated and are not reported on further. Samples were analyzed within 1 week of collection. Procedures for bacteriologic culture and identification were consistent with those previously published (Hogan et al., 1999). Milk samples were quickly thawed and shaken for 15 seconds and 0.1 mL of milk was plated on trypticase soy agar (TSA) with 5% sheep blood (BBL TSA II 5% SB agar; Becton, Dickinson and Co., Sparks MD). Plates were incubated at 36°C and examined after 24 and 48 hours of incubation. A culture was classified as contaminated if growth of 3 or more microbial species was observed (Dohoo et al., 2011). Non-contaminated cultures were classified as containing major pathogens, minor pathogens, or no growth. The major pathogens cultured included: *Staphylococcus aureus*, *Streptococcus spp., Trueperella pyogenes, Enterococcus spp., Pantoea spp.,* coliform bacteria (e.g., *Enterobacter aerogenes, Escherichia coli, Klebsiella spp.), Serratia marcescens,* and miscellaneous fungal species. Minor pathogens included: *Corynebacterium spp., Actinomyces spp., Acinetobacter spp*., and CNS. Infection classification criteria followed previously published methods (Anderson et al., 2010). Quarters were classified as infected if they contained any major pathogen species (MaP) at any concentration or minor pathogens

(MiP) at a concentration of 100 colonies/mL. Quarters containing both major and minor pathogens were classified as containing major pathogens and counted towards total number of infected quarters.

Milk Leukocyte Differential

Samples were analyzed using the AAD QScout Farm Lab (Advanced Animal Diagnostics, Inc., Morrisville, NC). Cell counts were reported as total leukocyte counts (TLC), which represented the sum of neutrophils (N), macrophages (M), and lymphocytes (L). This differs from the SCC in that epithelial cells are not included. The instrument also provides a "diagnosis" for each quarter, reported as "positive", "negative" or "borderline". To achieve normality of the data, transformation of cell counts was performed using the same equation used to convert SCC to somatic cell score (SCS = log base 2 (SCC /100,000) + 3), resulting in scores for each cell type (**NS** = neutrophil score, **MS** = macrophage score, and **LS** = lymphocyte score) and total leukocytes (**TLS** = total leukocyte score). Percentage of each cell type over the total leukocyte count was calculated and neutrophil percentage (**N%**), macrophage percentage (**M%**), and lymphocyte percentage (**L%**) were obtained.

Evaluation of the AAD QScout Farm Lab was done by comparison of diagnosis given by the machine with infection status according to microbiological culturing. Samples were analyzed using the early lactation setting of the AAD QScout Farm Lab which includes 18 diagnostic settings (1-18). Estimates of sensitivity (**Se**), specificity (**Sp**), and accuracy (**Ac**) were calculated for all settings and three threshold diagnostic settings were chosen for further analysis based on: default factory setting (setting 12, **diagA)**, highest specificity (setting 18, **diagB)**, and highest sensitivity (setting 2, **diagC**).

Cell thresholds that best represent infection status were obtained according to published methods (Youden, 1950), where a receiver operating characteristic curve was used to plot the true positive rate (**TPR**, sensitivity) and false positive rate (**FPR**, 1-specificity) of different cell thresholds for every cell type (**tTLC**, **tN**, **tM**, and **tL**).

Statistical Analyses

To estimate the effects of breed, lactation, day of sample collection, time of sampling, quarter location, animal, and week of sampling on cell score (TLS, NS, MS, LS), cell percentage (N%, M%, L%), MLD diagnosis setting (diagA, diagB, diagC), pathogen type occurrence (MaP, MiP), infection (INF), and infection according to cell thresholds the following model (Model I) was used:

$$\text{I) } y_{ijklmno} = \mu + Breed_i + Lactation_j + Day_k + TOD_l + Quarter_m + ID_{n(i)} + Week_o + e_{ijklmno}$$

Where $y_{ijklmno}$ is the investigated trait; μ is the overall mean; $Breed_i$ is the fixed effect of the *i*th class of breed (*i*=HO, JE); $Lactation_j$ is the fixed effect of the *j*th class of lactation number (*j*= 1, ≥2); Day_k is the fixed effect of the *k*th class of day of sample collection (*k*=4, 11); TOD_l is the fixed effect of the *l*th class of time of day (*l*= AM, PM); $Quarter_m$ is the fixed effect of the *m*th class of quarter location (*m*= left rear (LR), right rear (RR), left front (LF), right front (RF)); $ID_{n(i)}$ is the random effect of the *n*th class of animal within the *i*th class of breed [$a_c \sim N(0, \sigma^2_c)$]; $Week_o$ is the random effect of the *o*th class of week of sampling (1-48 weeks) [$u_w \sim N(0, \sigma^2_w)$]; $e_{ijklmno}$ is the random residual [$e \sim N(0, \sigma^2_e)$]. Model I was fitted using PROC GLIMMIX in SAS (SAS Version 9.4, SAS Institute Inc., Cary, NC), *F* values and *P* values for all effects, as well as least square means estimates were determined using the same procedure, where categorical traits were

transformed from the underlying liability scale to probability scale using previously published methods (Zwald et al., 2006). Statistical significance for all effects was considered present at $\alpha = 0.05$. Similar procedures were followed to estimate the additive genetic effect of the animal on the same traits listed previously. Data was analyzed using the following model (Model II):

$$\text{II) } y_{ijklmno} = \mu + Breed_i + Lactation_j + Day_k + TOD_l + Quarter_m + a_n + Ep_o + e_{ijklmno}$$

Where $y_{ijklmno}$ is the investigated trait; μ is the overall mean; $Breed_i$ is the fixed effect of the *i*th class of breed (*i*=HO, JE); $Lactation_j$ is the fixed effect of the *j*th class of lactation number (*j*= 1, ≥2); Day_k is the fixed effect of the *k*th class of day of sample collection (*k*=4, 11); TOD_l is the fixed effect of the *l*th class of time of day (*l*= AM, PM); $Quarter_m$ is the fixed effect of the *m*th class of quarter location (*m*= left rear (LR), right rear (RR), left front (LF), right front (RF)); a_n is the random additive genetic effect *n* of the animal following $a_c \sim N(0, \mathbf{A}\sigma^2_a)$]; Ep_o is the random permanent environmental effect *o* of each quarter in an individual animal, $u_p \sim N(0, \mathbf{I}\sigma^2_{pe})$; $e_{ijklmno}$ is the random residual, which follows $e \sim N(0, \mathbf{I}\sigma^2_e)$, where **I** is an identity matrix and **A** is the numerator relationship matrix build on a pedigree traced back nineteen generations. Parameters σ^2_a, σ^2_{pe} and σ^2_e were estimated using the THRGIBBS1F90 program (Tsuruta and Misztal, 2006) from the BLUPF90 family of programs. The random effect of week of sampling which served as our contemporary group explained none of the total phenotypic variance and therefore was excluded from the analysis in model II. Proportion of variance due the additive genetic effect, otherwise known as the narrow sense heritability (h^2) was calculated by dividing the variance component σ^2_a over the total phenotypic variance $\sigma^2_a + \sigma^2_{pe} + \sigma^2_e$. Data visualization was done with R Studio (v.0.99.903) (RStudio Team,

2015). Relative efficiency of selection for reduced INF using correlated traits, was calculated using the following equation:

$$\Delta G_c/\Delta G = \frac{h_x \; r_g}{h_y}$$

Where h_x is the heritability of the correlated trait, $r_{x.y}$ is the genetic correlation between the correlated trait and the trait for which genetic progress is desired (INF in our case), and h_y is the heritability of the trait for which genetic progress is desired.

RESULTS AND DISCUSSION

Microbiological Analysis

Culture classification and organism prevalence results for the remaining samples after removal of contaminated samples are reported in Table 1. After incubation of the samples, 197 (22.44%) cultures had growth of one or more microbial species, while 681 (77.56%) showed no growth (NG). This proportion of growth positive cultures to negative is higher than those reported in most studies with similar methodology, but is lower than values found in other studies (Jashari et al., 2016; Gonçalves et al., 2017). This may be due to a multitude of factors including: differences in sampling strategy, amount of quarters sampled, stage of lactation of sampled animals, criteria for growth classification, herd specific risk factors, etc. Of those samples that resulted in growth, 110 (55.84%) were classified as containing major pathogens (MaP). *Staphylococcus aureus* was the most prevalent major pathogen, followed by *Streptococcus dysgalactiae*. The remaining major pathogens were less prevalent and included: *Streptococcus uberis*, *Klebsiella spp.*, *Escherichia coli*, *Enterobacter aerogenes*, *Enterococcus spp.*, *Trueperella pyogenes*, *Pantoea spp.*, and *Serratia marcescens*. Minor pathogens (MiP) were present in 87 (44.16%) of growth positive cultures. CNS were the most prevalent

minor pathogens found, followed by *Corynebacterium spp.* Other minor pathogens found were *Actinomyces spp.* and *Acinetobacter spp.* Gonçalves et al. (2017) observed similar results for species of microorganisms isolated, frequency of specific pathogens and proportion of major pathogens to minor. Based upon our definition of infection, a total of 167 quarters (19.02%) were classified as infected (INF), while 711 (80.98%) were classified as not infected. Of those samples that were classified as infected (INF), 108 (64.67%) samples belonged to animals at day 4 of sample collection and 59 (35.33%) samples to animals at day 11 of sample collection, while 89 (53.29%) samples belonged to animals in their first lactation and 78 (46.71%) samples belonged to animals with 2 or more lactations. The comparability of our infection classification results with other studies is complicated by the fact that there is no widespread consensus of the pathogenicity of certain microorganisms, sampling and culturing methodology, and culture classification in mastitis research, therefore our classified scheme is one of several that could be used.

Milk Leukocyte Differential

Descriptive statistics for cell counts, cell scores, cell percentages, cell thresholds, and MLD diagnosis thresholds are given in Table 2. MLD diagnosis was obtained for all 987 quarter milk samples, but records for 111 samples were not kept due to incomplete information, these samples were classified as unknown and disregarded from diagnosis analyses. The results for the evaluation of the AAD QScout Farm Lab using the microbiological culturing infection status as the gold standard, were the following: the first threshold setting (diagA) had 44.72% Se, 92.69% Sp, and 84.55% Ac, the second threshold setting (diagB) had 34.93% Se, 95.40% Sp, and 84.63% Ac, and the third

threshold setting (diagC) had 60.27% Se, 83.60% Sp, and 79.46% Ac. Our estimates for Se are higher and for Sp are similar to those obtained by Godden et al. (2017) (Se = 12.7 to 39.1, Sp = 82.1 to 95.2), who performed a similar study evaluating the milk leukocyte differential test using the same machine and duplicate sampling for early lactation animals. Based on the values for sensitivity and specificity of the three settings the "best" threshold depends on whether your objective lies in decreasing the amount of quarters that are given treatment without having a true IMI (false positives) or the amount of quarters that are not treated while being infected (false negatives). This is the first study to obtain cell thresholds that maximize both Se and Sp and evaluate their use in mastitis detection and breeding applications as traits. The values for cell concentration that maximize the Se and Sp, and therefore best represent the difference between a positive or negative diagnosis were obtained by calculating the Youden's index (Youden, 1950) for various thresholds of every cell type. The selected cell concentration threshold was 162x10^3 cells/mL (Se=61.00%, Sp=80.03%) for TLC, 93 x10^3 cells/mL (Se=59.14%, Sp=83.48%) for N, 56 x10^3 cells/mL (Se=47.56%, Sp=89.06%) for M, and 47 x10^3 cells/mL (Se=56.70%, Sp=84.08%) for L. Estimates of Se and Sp among cell thresholds are very similar with tTLC having the highest Se (61.00%) and tM the highest Sp (89.06%). The ROC curve for each cell type thresholds is illustrated in Figure 1.

Estimation of F values, significance, and least square means

F value and *P* value results, as well as least square means estimates from model I are presented in Tables 3 and 4, respectively. The effect of breed was significant for all cell score traits considered, and for all cell threshold traits except tL. Jersey animals had on average higher cell scores and cell threshold infection rates; TLS (JE=3.24, HO=2.68,

P=0.012), NS (JE=2.23, HO=1.64, P=0.015), MS(JE=1.06, HO=0.64, P=0.033), LS (JE=1.02, HO=0.42, P=0.009), tTLC(JE=0.28, HO=0.15, P=0.020), tN (JE=0.25, HO=0.13, P=0.025), and tM(JE=0.16, HO=0.08, P=0.020). One possible explanation for this phenomenon is the difference in milk volume produced by these two breeds, which would cause higher concentration of somatic cells in Jerseys. Breed has previously been found to be a significant factor for milk yield, milk and protein composition traits, and milk coagulation properties (Bobbo et al., 2017; Stocco et al., 2017). The effect of lactation was significant for MaP occurrence and INF. Similar to our study, Pilla et al. (2012) found that lactation number was not significant for any of the cell percentages studied (N%, M%, or L%). Animals in their first lactation had a higher MaP (1=0.14, ≥2=0.03, P=0.0002) and INF (1=0.26, ≥2=0.11, P=0.0007) when compared to animals with multiple lactations. These results are in accordance with findings by Compton et al. (2007), who found that the risk of CM in heifers is three times that of older cows in the early stages of lactation, and also in accordance with Barkema et al. (1998), who found that the incidence rate of CM was higher in heifers than in cows. The effect of day of sample collection was significant for all traits considered in this study except for N%, MiP, and a positive diagnosis using diagB. Animals in the 4th day of sample collection had on average higher cell scores, L%, cell threshold infection rate, MaP, INF, and rate of positive diagnosis at diagA and diagC, than animals at the 11th day of sample collection; TLS (4=3.36, 11=2.56, P<0.0001), NS (4=2.35, 11=1.51, P<0.0001), MS (4=1.21, 11=0.49, P<0.0001), LS (4=1.19, 11=0.26, P<0.0001), L% (4=0.23, 11=0.22, P=0.0150), tTLC (4=0.30, 11=0.14, P<0.0001), tN (4=0.25, 11=0.13, P<0.0001), tM (4=0.16, 11=0.08, P=0.0034), tL (4=0.24, 11=0.12, P<0.0001), MaP (4=0.09, 11=0.05, P=0.0256), INF (4=0.21, 11=0.14, P=0.0095), diagA (4=0.09, 11=0.06, P=0.0498), and diagC

(4=0.25, 11=0.12, P=0.0022). These results for cell score, cell threshold, and diagnosis traits are expected as it is widely known that the level of somatic cells, specifically neutrophils, is high at parturition and declines gradually during the first weeks of lactation, even for non-infected animals (Dohoo, 1993). The elevated occurrence of major pathogens and infection might be due to the persistence of dry period infections and/or an increased risk of infection in fresh animals by opportunistic pathogens. Animals in the 11th day of sample collection had higher M% than those at the 4th day; M% (4=0.25, 11=0.28, P=0.0017). The changes in cell proportions as they relate to SCC increase have been heavily investigated, Pilla et al. (2012) found that macrophage percentage was lower in early lactation and found the effect of day of sample collection to be significant for macrophage percentage, meanwhile Damm et al. (2017) found decreasing trend of macrophage percentage as SCC increased. Therefore, our results can be due to an increase in macrophage percentage as a factor of the previously mentioned decline of N and L after the first days of lactation. The effect of time of sampling was significant for NS, N%, and M%. Samples taken in the morning resulted in a higher M%, meanwhile sampling in the evening resulted in higher NS and N%; M% (AM=0.28, PM=0.25, P=0.0130), NS (AM=1.73, PM=2.13, P=0.0315), and N% (AM=0.49, PM=0.52, P=0.0026). These results reflect changes in cell proportion that occur during the day and are consistent with the observed diurnal variation of cells by Riekerink et al. (2007), specifically changes in proportions of neutrophils and macrophages post milking. Finally, the effect of quarter position was significant for M%, L%, MiP, and INF. For these traits, at least one quarter had an estimate that differed significantly from one or more quarters; M% (LF=0.29, LR=0.25, RF=0.27, RR=0.25, P=0.0009), L% (LF=0.21, LR=0.24, RF=0.22, RR=0.24, P<0.0001), MiP (LF=0.08, LR=0.10, RF=0.05, RR=0.17, P=0.0030),

and INF (LF=0.15, LR=0.17, RF=0.14, RR=0.24, P=0.0419). Quarter position has been found in previous studies not to be significantly associated with cell percentages (Pilla et al., 2012, 2013) or with clinical mastitis occurrence (Hammer et al., 2012). Our results seem to indicate that there is some effect, although its implications for mastitis research and possible biological explanations are yet to be elucidated.

Variance components, phenotypic and genetic correlations, and correlated selection.

Variance component and heritability results for all traits analyzed using Model II are presented in Table 5. Cell score traits had moderate heritability that ranged from 0.37 to 0.40; TLS and NS both had an estimate of 0.40, followed by estimates of 0.38 and 0.37 for MS and LS respectively. Cell percentage traits had low to moderate heritability that ranged from 0.27 to 0.29; N% had an estimate of 0.27 and M% an estimate of 0.29. Due to poor convergence, variance component and heritability estimates could not be calculated for L%. Cell threshold traits had moderate to high heritability that ranged from 0.48 to 0.57; tTLC had an estimate of 0.57, both tN and tL had an estimate of 0.51, and tM an estimate of 0.48. MLD diagnosis traits had moderate to high heritability, positive diagnosis had an estimate of 0.50, 0.48, and 0.53 for diagA, diagB, and diagC respectively. No previous study that we could find has looked at the estimation of genetic parameters for cell type traits or diagnosis derived from MLD, therefore comparison of our estimates with other studies is not possible. However, estimates for the heritability of SCS, a proxy for our TLS trait, have been previously reported to be 0.11 for early lactation (Koeck et al., 2012), 0.18 for general SCS (CDN, 2016), and 0.20 and 0.23 for lactation average SCC at 170 and 300 days respectively (Sorensen et al., 2009). Our estimate of 0.40 for TLS is significantly higher than most estimates in previously

published literature for SCS, this is most likely due to our experimental design (small sample size and repeated records for animals). Variance component and heritability estimates for MaP and MiP could not be calculated due to low convergence. Lastly, INF had a moderate heritability of 0.41. Comparing our INF heritability estimate is hindered by the scope of our study which was early lactation and our definition of infection as a trait. Nevertheless, our estimate is higher than those previously reported for similar traits, for example, Sørensen et al. (2009) reported an estimate for unspecified mastitis of 0.14. It is our belief that the inflated nature of the heritability estimates presented in this study is mainly due to low sample size.

Estimates for the phenotypic and genetic correlations between traits related to somatic cell measures are presented in Table 6. Cell score traits (TLS, NS, MS, and LS) exhibited highly positive phenotypic and genetic correlations between themselves, ranging from 0.79 to 0.98 and from 0.81 to 0.99 respectively. This implies that all cell types increase jointly with an increase of TLS and that there is a shared genetic architecture responsible for the immunological response. Correlations between cell percentage traits (N%, M%, and L%) were phenotypically moderate to high and negative, ranging from -0.30 to -0.71, and genetically highly negative, ranging from -0.82 to -0.85. The estimates between N% and L% were not significant. Schwartz et al. (2011) found statistically significant negative phenotypic correlations between SCC and both L% and M%, and a significant positive phenotypic correlation between SCC and N%, all of which is in accordance with our results. Phenotypic correlations between cell score and cell percentage traits were low to moderate and positive for N% and all cell score traits, ranging from 0.10 to 0.52, and for L% and LS, which had an estimate of 0.18. M% had moderately negative phenotypic correlations with cell score traits, ranging from -0.34 to -

0.48. Phenotypic estimates between M% and MS, L% and TLS, and L% and NS were not significant. Genetic correlations were moderate to high and positive between N% and cell score traits, ranging from 0.53 to 0.65, and were moderate and negative between M% and cell score traits, ranging from -0.43 to -0.58. Genetic correlation estimates between MS and all cell percentage traits, and between L% and all cell score traits were not significant. Overall, cell score traits are very highly correlated to each other and with N% suggesting their interchangeable use in selection.

Estimates for the phenotypic and genetic correlations between infection and all other traits, and relative efficiency of selection for reduced infection on correlated traits are presented in table 7. Phenotypic and genetic correlations with cell score traits were moderate and positive ranging from 0.41 to 0.48 phenotypically and from 0.39 to 0.46 genetically. Correlations with cell percentage traits were low to moderate, being positively correlated with N% and negatively correlated with M%. Estimates were 0.30 and -0.28 phenotypically, and 0.29 and -0.24 genetically, for N% and M% respectively. Correlations with all cell threshold traits were moderate and positive, ranging from 0.52 to 0.56 phenotypically and from 0.48 to 0.50 genetically. A positive diagnosis in all three settings was moderate to highly and positively correlated, with estimates ranging from 0.55 to 0.65 phenotypically and from 0.51 to 0.61 genetically. Estimates for relative efficiency of selection for reduced infection on correlated traits range from -0.20 for M% to 0.69 for diagC. These results suggest that selection using MLD traits, specifically MLD diagnosis traits might prove useful, considering the high cost of diagnosing mastitis using microbial culturing.

CONCLUSIONS

Milk leukocyte differential is a tool that has been recently developed to diagnose mastitis using the changes in somatic cell populations and overall proportions to do so. MLD has been found to have high specificity, correctly diagnosing quarters that are not infected, although our results and previously published studies have consistently shown that this method only has moderate sensitivity. This study has found that for the traits derived from MLD, there are significant differences at the quarter level when it comes to breed, lactation, day of sample collection, time of sampling, and quarter position. Estimates of genetic parameters such as low to moderate heritability estimates for cell score and cell percentage traits, and moderate to high estimates for cell threshold, MLD diagnosis, and infection traits are promising. Results for estimated genetic correlations with infection and relative efficiency of selection for reduced infection, show that the traits derived from MLD have merit for inclusion in selection indices for decreased mastitis susceptibility. Further research must be done validating results of this study in different populations and with pathogen specific infection before such implementations are possible.

Table 1. Milk culture classification and organism prevalence[1]

Classification	#	%[2]	%[3]	Mean TLC (cells/mL)	Mean N (cells/mL)	Mean M (cells/mL)	Mean L (cells/mL)
No Growth	681	77.56		129.80 x10^3	70.98 x10^3	29.53 x10^3	29.58x10^3
Major Pathogens	110	12.53	55.84	1,406.00 x10^3	988.30x10^3	159.30 x10^3	257.7 x10^3
Staphylococcus aureus	47	5.35	23.86				
Staphylococcus aureus dual infections [4]	5	0.57	2.54				
Streptococcus dysgalactiae	26	2.96	13.20				
Streptococcus dysgalactiae dual infections [5]	7	0.80	3.55				
Streptococcus uberis	3	0.34	1.52				
Escherichia coli + *Enterococcus spp.*	1	0.11	0.51				
Enterobacter aerogenes	1	0.11	0.51				
Enterococcus spp.	1	0.11	0.51				
Pantoea spp.	1	0.11	0.51				
Serratia marcescens	2	0.23	1.02				
Trueperella pyogenes + *Corynebacterium spp.*	1	0.11	0.51				
Enterococcus spp. + CNS	1	0.11	0.51				
Fungi	14	1.59	7.11				
Minor Pathogens	87	9.91	44.16	201.70 x10^3	105.40 x10^3	47.56 x10^3	38.05 x10^3
Actinomyces spp.	1	0.11	0.51				
CNS	55	6.26	27.92				
CNS dual infections [6]	4	0.46	2.03				
Corynebacterium spp.	27	3.08	13.71				
Total	878	100.00	100.00				

[1] TLC = Total Leukocyte Count; N = Neutrophil Count; M = Macrophage Count; L = Lymphocyte Count.
[2] Percent calculated on the total amount of non-contaminated cultures (N=878).
[3] Percent calculated on the total amount of growth positive cultures (N=197).
[4] *Staphylococcus aureus* in combination with: *Escherichia coli* (1), Trueperella *pyogenes* (1), *Corynebacterium spp.* (1), and *Streptococcus dysgalactiae* (2).
[5] *Streptococcus dysgalactiae* in combination with: *Escherichia coli* (1), Serratia *marcescens* (1), *Klebsiella spp.* (1), and CNS (4).
[6] CNS in combination with: *Acinetobacter spp.* (2) and *Corynebacterium spp.* (2).

Table 2. Descriptive Statistics for milk leukocyte differential and diagnosis traits by day of sample collection.

Cell Measure:[1]	Mean		Median		Min value		Max value		Standard Deviation	
	Day 4	Day 11	Day 4	Day 11	Day 4	Day 11	Day 4	Day 11	Day 4	Day 11
Cell Count[2]										
TLC	368.80	227.18	104.00	59.50	15.00	7.00	12149.00	17250.00	1153.88	1019.03
N	235.73	144.24	50.00	28.00	5.00	3.00	9040.00	12750.00	851.75	747.12
M	60.78	33.18	27.00	16.00	2.00	0.00	1614.00	984.00	135.00	72.32
L	72.43	49.71	24.00	12.00	2.00	1.00	2152.00	3517.00	189.19	211.49
Cell Scores										
TLS	3.35	2.53	3.06	2.25	0.26	-0.64	9.92	10.43	1.69	1.66
NS	2.37	1.50	2.00	1.16	-1.32	-2.05	9.50	9.99	1.83	1.83
MS	1.18	0.44	1.11	0.36	-2.64	-10.29	7.01	6.30	1.59	1.50
LS	1.17	0.23	0.94	-0.06	-2.64	-3.64	7.43	8.13	1.72	1.85
Cell Percentages										
N%	51.95%	50.82	51.92%	50.65%	9.63%	13.79%	77.88%	89.09%	10.43%	11.98%
M%	25.13%	27.56%	23.75%	25.81%	4.63%	0.00%	74.13%	83.33%	12.00%	14.70%
L%	24.11%	22.02%	22.74%	21.45%	0.40%	2.22%	84.18%	63.08%	8.24%	8.45%
Cell Thresholds[3]										
tTLC	0.34	0.18								
tN	0.30	0.17								
tM	0.22	0.11								
tL	0.27	0.13								
MLD Diagnosis[3]										
DiagA	0.15	0.11								
DiagB	0.10	0.07								
DiagC	0.27	0.16								

[1] TLC = Total Leukocyte Count; N = Neutrophil Count; M = Macrophage Count; L = Lymphocyte Count; TLS = Total Leukocyte Score; NS = Neutrophil Score; MS = Macrophage Score; LS = Lymphocyte Score; N% = Neutrophil Percentage; M% = Macrophage Percentage; L% = Lymphocyte Percentage; tTLC = Total Leukocyte Count Threshold; tN = Neutrophil Threshold; tM = Macrophage Threshold; tL = Lymphocyte Threshold; DiagA= Positive Diagnosis Threshold Setting A; DiagB = Positive Diagnosis Threshold Setting B; DiagC= Positive Diagnosis Threshold Setting C.
[2] Unit for cell count is x10^3 cells/mL.
[3] Occurrence of infection according to threshold.

Table 3. ANOVA results for model I

Trait[1]	Fixed Effects				
	Breed	Day of Sample Collection	Lactation	Time of Sampling	Quarter
Cell Score					
TLS	6.47**	79.5***	0.8	2.86	0.68
NS	6.09**	76.53***	0.64	4.64*	0.75
MS	4.64*	71.93***	0.34	0.14	0.61
LS	7.06**	90.03***	0.91	2.49	2.53
Cell Percentages					
N%	1.41	2.88	0	9.10**	0.72
M%	1.37	9.95**	0.48	6.20*	5.56***
L%	0.09	5.95*	0.21	0.07	11.39***
Cell Thresholds					
tTLC	5.54*	25.6***	1.8	0.19	1.0
tN	5.16*	16.4***	2.01	0.56	1.41
tM	5.41*	8.66**	0.43	0.71	0.48
tL	3.8	19.17***	1.28	0.02	2.1
Microbial Culturing					
MaP	1.78	5.0*	13.87***	0.51	0.23
MiP	0.07	0.8	1.39	1.97	4.69**
INF	0.67	6.75**	11.71***	0	2.75*
MLD Diagnosis					
DiagA	1.95	3.86*	0.86	2.31	0.38
DiagB	2.98	2.30	0.27	0.51	0.15
DiagC	2.16	18.28***	0.27	0.42	1.37

[1] TLS = Total Leukocyte Score; NS = Neutrophil Score; MS = Macrophage Score; LS = Lymphocyte Score; N% = Neutrophil Percentage; M% = Macrophage Percentage; L% = Lymphocyte Percentage; tTLC = Total Leukocyte Count Threshold; tN = Neutrophil Threshold; tM = Macrophage Threshold; tL = Lymphocyte Threshold; MaP = Major Pathogen Occurrence; MiP = Minor Pathogen Occurrence; INF = Infection; DiagA= Positive Diagnosis Threshold Setting A; DiagB = Positive Diagnosis Threshold Setting B; DiagC = Positive Diagnosis Threshold Setting C.
* P<0.05; ** P<0.01; *** P<0.001

Table 4. Least Squares Means (LSMEANS) results for model I (SE within parentheses)

	Least Squares Means											
	Breed		Lactation		Day of Sample Collection		Time of Sampling		Quarter			
Trait[1]	Holstein	Jersey	1st Lactation	≥2 Lactations	Day 4	Day 11	AM	PM	Left Front Quarter	Left Rear Quarter	Right Front Quarter	Right Rear Quarter
Cell Score												
TLS	2.68(0.15)	3.24(0.19)	3.06(0.19)	2.86(0.15)	3.36(0.14)	2.56(0.14)	2.81(0.18)	3.11(0.12)	2.86(0.15)	2.97(0.15)	3.01(0.15)	3.01(0.15)
NS	1.64 (0.16)	2.23(0.20)	2.03(0.20)	1.84(0.16)	2.35(0.15)	1.51(0.15)	1.73(0.20)	2.13(0.13)	1.82(0.16)	1.93(0.16)	1.98(0.16)	2.00(0.16)
MS	0.64(0.14)	1.06(0.17)	0.91(0.17)	0.79(0.14)	1.21(0.13)	0.49(0.13)	0.82(0.17)	0.88(0.12)	0.88(0.14)	0.78(0.14)	0.92(0.14)	0.82(0.14)
LS	0.42(0.16)	1.02(0.20)	0.83(0.20)	0.62(0.16)	1.19(0.14)	0.26(0.15)	0.57(0.20)	0.87(0.13)	0.53(0.16)	0.80(0.16)	0.71(0.16)	0.86(0.16)
Cell Percentages												
N%	0.50(0.01)	0.51(0.01)	0.51(0.01)	0.51(0.01)	0.51(0.01)	0.50(0.01)	0.49(0.01)	0.52(0.01)	0.50(0.01)	0.50(0.01)	0.51(0.01)	0.51(0.01)
M%	0.27(0.01)	0.26(0.01)	0.26(0.01)	0.27(0.01)	0.25(0.01)	0.28(0.01)	0.28(0.01)	0.25(0.01)	0.29(0.01)	0.25(0.01)	0.27(0.01)	0.25(0.01)
L%	0.23(0.01)	0.23(0.01)	0.23(0.01)	0.22(0.01)	0.23(0.01)	0.22(0.01)	0.23(0.01)	0.23(0.01)	0.21(0.01)	0.24(0.01)	0.22(0.01)	0.24(0.01)
Cell Thresholds												
tTLC	0.15(0.03)	0.28(0.06)	0.25(0.05)	0.17(0.04)	0.3(0.04)	0.14(0.03)	0.2(0.05)	0.22(0.03)	0.17(0.04)	0.21(0.04)	0.23(0.05)	0.23(0.05)
tN	0.13(0.03)	0.25(0.05)	0.22(0.05)	0.15(0.03)	0.25(0.04)	0.13(0.03)	0.17(0.05)	0.20(0.03)	0.14(0.03)	0.19(0.04)	0.21(0.04)	0.21(0.04)
tM	0.08(0.02)	0.16(0.04)	0.13(0.04)	0.10(0.03)	0.16(0.03)	0.08(0.02)	0.1(0.03)	0.13(0.03)	0.10(0.03)	0.13(0.03)	0.10(0.03)	0.13(0.03)
tL	0.13(0.03)	0.22(0.05)	0.20(0.04)	0.14(0.03)	0.24(0.03)	0.12(0.03)	0.17(0.04)	0.18(0.03)	0.12(0.03)	0.18(0.04)	0.19(0.04)	0.21(0.04)
Microbial Culturing												
MaP	0.05(0.02)	0.09(0.03)	0.14(0.04)	0.03(0.01)	0.09(0.02)	0.05(0.02)	0.06(0.03)	0.08(0.02)	0.07(0.02)	0.06(0.02)	0.06(0.02)	0.08(0.03)
MiP	0.1(0.02)	0.09(0.02)	0.11(0.03)	0.08(0.02)	0.09(0.02)	0.11(0.02)	0.12(0.03)	0.08(0.01)	0.08(0.02)	0.10(0.03)	0.05(0.02)	0.17(0.03)
INF	0.15(0.03)	0.19(0.04)	0.26(0.05)	0.11(0.02)	0.21(0.03)	0.14(0.03)	0.17(0.04)	0.17(0.03)	0.15(0.03)	0.17(0.04)	0.14(0.03)	0.24(0.04)
MLD Diagnosis												
DiagA	0.06(0.02)	0.09(0.03)	0.09(0.03)	0.06(0.02)	0.09(0.02)	0.06(0.02)	0.05(0.02)	0.10(0.02)	0.06(0.02)	0.08(0.02)	0.08(0.02)	0.07(0.02)
DiagB	0.05(0.02)	0.09(0.03)	0.08(0.03)	0.06(0.02)	0.08(0.02)	0.06(0.02)	0.06(0.03)	0.08(0.02)	0.06(0.02)	0.07(0.02)	0.08(0.03)	0.07(0.02)
DiagC	0.14(0.04)	0.22(0.06)	0.19(0.06)	0.16(0.04)	0.25 (0.05)	0.12(0.03)	0.16(0.05)	0.19(0.04)	0.14(0.04)	0.17(0.05)	0.21(0.05)	0.21(0.05)

[1] TLS = Total Leukocyte Score; NS = Neutrophil Score; MS = Macrophage Score; LS = Lymphocyte Score; N% = Neutrophil Percentage; M% = Macrophage Percentage; L% = Lymphocyte Percentage; tTLC = Total Leukocyte Count Threshold; tN = Neutrophil Threshold; tM = Macrophage Threshold; tL = Lymphocyte Threshold; MaP = Major Pathogen Occurrence; MiP = Minor Pathogen Occurrence; INF = Infection; DiagA= Positive Diagnosis Threshold Setting A; DiagB = Positive Diagnosis Threshold Setting B; DiagC = Positive Diagnosis Threshold Setting C.

Table 5. Variance component and heritability (h^2) results for model II (95% CI within parentheses)

Trait[1]	Variance Components: Additive Variance	Cow Quarter Permanent Environmental Variance	Residual Variance	Total Phenotypic Variance	h^2
Cell Score					
TLS	1.16 (0.76, 1.61)	0.62 (0.43, 0.78)	1.12 (0.97, 1.26)	2.90 (2.47, 3.35)	0.40 (0.31, 0.49)
NS	1.06 (0.71, 1.48)	0.56 (0.40, 0.73)	1.02 (0.0.87, 1.14)	2.64 (2.27, 3.06)	0.40 (0.31, 0.49)
MS	0.50 (0.33, 0.71)	0.23 (0.17, 0.31)	0.56 (0.50, 0.65)	1.31 (1.14, 1.52)	0.38 (0.29, 0.47)
LS	0.65 (0.42, 0.90)	0.35 (0.25, 0.46)	0.75(0.65, 0.83)	1.75 (1.52, 2.02)	0.37 (0.28, 0.46)
Cell Percentages					
N%	$0.39x10^{-2}$ ($0.30x10^{-2}$, $0.60 x10^{-2}$)	$0.25 x10^{-3}$ (0, $0.10 x10^{-2}$)	$0.10x10^{-1}$ ($0.90 x10^{-2}$, $0.11x10^{-1}$)	$0.15x10^{-1}$ ($0.13x10^{-1}$, $0.16x10^{-1}$)	0.27 (0.19, 0.35)
M%	$0.54x10^{-2}$ ($0.40 x10^{-2}$, $0.80 x10^{-2}$)	$0.35x10^{-3}$ (0, $0.10 x10^{-2}$)	$0.13 x10^{-1}$ ($0.12 x10^{-1}$, $0.14 x10^{-1}$)	$0.19 x10^{-1}$ ($0.16 x10^{-1}$, $0.21 x10^{-1}$)	0.29 (0.21, 0.37)
L%	Not Converged	Not Converged	Not Converged	Not Converged	Not Converged
Cell Thresholds					
tTLC	2.75 (1.67, 3.98)	1.07 (0.34, 1.88)	1.00 (0.88, 1.16)	4.82 (3.37, 6.40)	0.57 (0.43, 0.70)
tN	1.89 (1.04, 2.76)	0.78 (0.20, 1.38)	1.01 (0.87, 1.15)	3.67 (2.57, 4.78)	0.51 (0.37, 0.66)
tM	1.58 (0.86, 2.26)	0.69 (0.15, 1.35)	1.01 (0.87, 1.16)	3.27 (2.29, 4.28)	0.48 (0.33, 0.62)
tL	1.92 (1.14, 2.78)	0.84 (0.23, 1.53)	1.01 (0.87, 1.15)	3.77 (2.60, 4.82)	0.51 (0.37, 0.65)
Microbial Culturing					
MaP	Not Converged	Not Converged	Not Converged	Not Converged	Not Converged
MiP	Not Converged	Not Converged	Not Converged	Not Converged	Not Converged
INF	1.48 (0.80, 2.29)	1.12 (0.33, 1.99)	1.01 (0.87, 1.15)	3.60 (2.58,4.94)	0.41 (0.27, 0.56)
MLD Diagnosis					
DiagA	1.97 (0.99, 3.09)	0.90 (0.19, 1.69)	1.00 (0.87, 1.15)	3.90 (2.50, 5.20)	0.50 (0.32, 0.66)
DiagB	1.71 (0.79, 2.77)	0.87 (0.03, 1.87)	1.01 (0.87, 1.15)	3.59 (2.23, 5.20)	0.48 (0.30, 0.64)
DiagC	2.42 (1.35, 3.71)	1.10 (0.35, 1.93)	1.01 (0.87, 1.16)	4.52 (3.12, 6.13)	0.53 (0.38, 0.67)

[1] TLS = Total Leukocyte Score; NS = Neutrophil Score; MS = Macrophage Score; LS = Lymphocyte Score; N% = Neutrophil Percentage; M% = Macrophage Percentage; L% = Lymphocyte Percentage; tTLC = Total Leukocyte Count Threshold; tN = Neutrophil Threshold; tM = Macrophage Threshold; tL = Lymphocyte Threshold; MaP = Major Pathogen Occurrence; MiP = Minor Pathogen Occurrence; INF = Infection; DiagA= Positive Diagnosis Threshold Setting A; DiagB = Positive Diagnosis Threshold Setting B; DiagC = Positive Diagnosis Threshold Setting C.

Table 6. Phenotypic (above diagonal) and genetic (below diagonal) correlations between somatic cell measures [1,2]

	TLS	NS	MS	LS	N%	M%	L%
TLS		**0.98**	**0.89**	**0.96**	**0.38**	**-0.33**	-0.03
NS	**0.99**		**0.84**	**0.95**	**0.52**	**-0.44**	-0.03
MS	**0.95**	**0.85**		**0.79**	**0.10**	0.07	**-0.15**
LS	**0.99**	**0.98**	**0.81**		**0.40**	**-0.48**	**0.18**
N%	**0.53**	**0.65**	0.20	**0.57**		**-0.71**	-0.01
M%	**-0.43**	**-0.52**	-0.02	**-0.58**	**-0.85**		**-0.30**
L%	-0.11	-0.13	-0.38	-0.02	-0.11	**-0.82**	

[1] TLS = Total Leukocyte Score; NS = Neutrophil Score; MS = Macrophage Score; LS = Lymphocyte Score; N% = Neutrophil Percentage; M% = Macrophage Percentage; L% = Lymphocyte Percentage.
[2] Estimates in boldface are statistically significant.

Table 7. Phenotypic (r_{ph}) and genetic correlations (r_g) with INF and relative efficiency of selection ($\Delta G_c/\Delta G$) for INF on correlated trait.

Trait	r_{ph}	r_g	[a]$\Delta G_c/\Delta G$ [2]
TLS	0.48	0.43	0.42
NS	0.48	0.43	0.42
MS	0.41	0.39	0.38
LS	0.46	0.46	0.44
N%	0.30	0.29	0.24
M%	-0.28	-0.24	-0.20
tTLC	0.52	0.48	0.57
tN	0.54	0.50	0.56
tM	0.56	0.49	0.53
tL	0.53	0.49	0.55
DiagA	0.55	0.51	0.56
DiagB	0.65	0.60	0.65
DiagC	0.63	0.61	0.69

[1] TLS = Total Leukocyte Score; NS = Neutrophil Score; MS = Macrophage Score; LS = Lymphocyte Score; N% = Neutrophil Percentage; M% = Macrophage Percentage; tTLC = Total Leukocyte Count Threshold; tN = Neutrophil Threshold; tM = Macrophage Threshold; tL = Lymphocyte Threshold; DiagA= Positive Diagnosis Threshold Setting A; DiagB = Positive Diagnosis Threshold Setting B; DiagC = Positive Diagnosis Threshold Setting C.

[2] $\Delta G_c/\Delta G = \frac{h_x \bullet r_g}{h_y}$

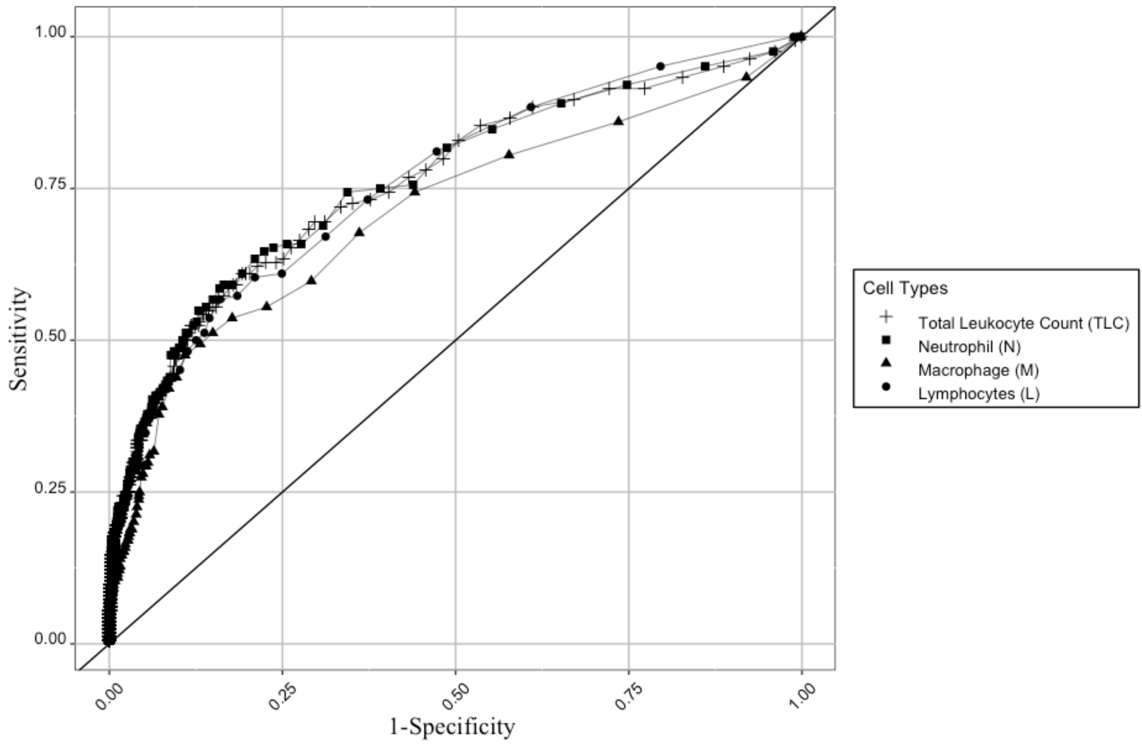

Figure 1. Combined ROC curves for cell concentration thresholds.

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CHAPTER 3

Thesis Conclusions

The importance of the research presented in this dissertation is to examine the use of milk leukocyte differential technology to improve mastitis detection and treatment, as well as serve the purpose of providing novel traits to which selection pressure can be applied to increase mastitis resistance. In the first chapter, we established the published literature relevant to the definition of intramammary infection, economic effects of mastitis, responsible pathogens, immune response, mastitis detection methods, and what has been done in terms of selection towards resistance. In the second chapter, we examined the viability of traits derived from MLD technology (cell score, cell percentage, cell threshold, and MLD diagnosis traits) for use in mastitis monitoring and in selection for resistant animals. Analysis of the ability of detection of infected quarters for cell and diagnostic thresholds showed low to moderate sensitivity and high specificity for diagnostic thresholds, and moderate sensitivity and high specificity for cell thresholds. For the effects analyzed we found differences in breed, lactation, day of sample collection, time of sample collection, and quarter position for one or more traits in our study. In the estimation of genetic parameters, we found low to moderate heritability estimates for cell score and percentage traits, and moderate estimates for cell threshold, MLD diagnosis, and infection traits. The strength and direction of the genetic correlations within and between cell score and cell percentage traits were in accordance with the nature of immune response. Finally, genetic correlations between analyzed MLD traits and infection as well as estimates for the relative efficiency of selection for decreased intramammary infection using MLD traits indicate the potential inclusion of these traits in selection programs for mastitis disease resistance. Our hope is that the work done in this dissertation serves to drive further research into the use of novel traits derived from cell differentiation for selection against intramammary infection.