GENETIC AND MATERNAL FACTORS UNDERLYING EARLY MILK PRODUCTION AND THEIR INFLUENCE ON CALF HEALTH

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ACADEMIC ABSTRACT

The quality of early milk produced by dams is affected by various factors (i.e. breed, age, parity, environment, nutrition, management). The impact of these factors on the quality of milk then have subsequent effects on calf health and development**.** Producers are responsible for following guidelines in order to ensure that they feed calves optimal quality milk in order to produce a healthy animal. They can also regulate factors such as environment and nutrition of the dam in order to produce better quality early milk. However, even after maximizing these factors there is still high mortality rate among pre-weaned calves, therefore, other factors such as mode of birth and genetics need to be studied to determine impacts on early milk quality and make further improvements to calf health and decrease mortality. Two experiments were conducted in order to study the effects of maternal and genetic factors on early milk production and to determine relationships that exist with calf health. The objective of the first study was to determine the effects that the mode of delivery had on early milk composition, and on the rumen microbiome of calves. We hypothesized that mode of birth would impact early milk composition, and, in turn, influence the microbial phyla in the calf gut. The second study had three objectives: 1) establish phenotypic relationships between colostrum composition traits, milk production traits, and calf health, 2) determine impact of breed and season on colostrum production and 3)) elucidate the genetic parameters (i.e. heritability, genotypic, and phenotypic correlations) among colostrum production and milk production We hypothesized that colostrum composition and production differ among breeds and by season and that individual components

influence calf health. Additionally, we hypothesized that colostrum quality traits (i.e. Brix score and volume) are heritable.

For the first study Charolaise (**CHAR;** n = 23) and Angus (**ANG;** n = 15) dams were divided into two experimental groups; dams underwent vaginal (**VD;** n= 25) or cesarean (**CD;** n= 13) deliveries. Early milk samples were collected and quantified for protein, lactose, somatic cell count, and fatty acid concentrations. After parturition calves were separated based on dams experimental group. Rumen fluid was collected from calves on d 1, 3, and 28 post-partum. Extracted DNA from fluid were used for metagenomic sequencing (ANG calves, n=11; CHAR calves, n=13). Samples were run on the HiSeq 2500 platform as paired end reads according to Ilumina's standard sequencing protocol. A regression analysis was done in SAS using PROC GLM and regressing mode of birth on milk components for d 1,3, and 28. After, milk components found to be significantly impacted by mode of birth were regressed against microbial counts. Results showed that VD dams were more likely to have increased ($P \leq 0.05$) protein, solids non-fat, and lactose on d 1 and 3, but decreased ($P < 0.05$) urea concentrations. Similarly, short, medium, and long-chain fatty acids were increased ($P \leq 0.05$) in VD d 3 milk. Changes in true protein elicited a decrease ($P \leq 0.05$) in rumen fluid Actinobacteria and Proteobacteria; whereas, both solids non-fat and lactose were associated with an increased ($P \leq 0.05$) response in d 1 transition milk. No significant results for d 28 of sampling were observed. Based on our results we suggest that mode of birth influences protein concentrations in early milk. However, only a slight impact on the overall dynamics of the calf rumen was observed with the microbiome remaining relatively stable on the phyla level in response to changes in protein concentration.

The second study looked into relationships between colostrum composition traits, management practices, and calf health, as well as determined heritability and genetic correlations for colostrum quality traits. Values for test-day milk, protein, fat, and somatic cell count (**SCS**) for Holstein (**HO**, n= 250) and Jersey (**JE**, n=289) cows were obtained from the Animal Genomic and Improvement laboratory server at the USDA. Brix score, colostrum weight, dam age, parity, and 3-month season of calving were also recorded. After, colostrum samples from JE cows were sent to DHIA where compositional measurements were obtained (i.e. true protein, fat, lactose, SCS, solid non-fats). Lactoferrin concentration for JE colostrum samples was also determined via ELISA. Calf blood samples were collected within 72 h post-partum and total serum protein (**TSP**) quantified to determine success of passive immunity transfer. Additionally, farm staff were instructed to record colostrum source for 1st feeding (i.e. dam, mix, other), freshness for 1st feeding (frozen vs fresh), Brix score of colostrum fed, volume of colostrum fed, and birth weight. A PROC Mixed with LSMEANS was performed in SAS to determine relationships between colostrum components, test day components, and quality traits for season, breed, and the interaction between season and breed. Also, PROC Mixed with LSMEANS was used to determine relationships of calf health with environment, management, and colostrum components. Additionally, a Pearson correlation was used to determine relationships between colostrum components and quality traits. Results for Holstein and Jersey showed that both colostrum Brix and volume ($P < 0.001$) differed by breed. Colostrum volume ($P < 0.001$), lactose ($P < 0.001$), and lactoferrin ($P = 0.002$) varied significantly by season. Additionally, test day milk ($P = 0.046$), fat ($P = 0.012$), and protein ($P = 0.003$) varied significantly by season. Moreover, a significant season and breed interaction ($P = 0.028$) was observed solely for colostrum volume. Calf health models indicated that TSP, colostrum total protein and solid non-

fats impacted incidence of respiratory illness, but no factor significantly impacted incidence of scours. Results for Pearson correlation indicated strong correlations between true protein and solid non-fats and Brix (r = 0.99; 0.86). Lactoferrin also had moderate negative correlations with volume and lactose (r = -0.35; -0.33). Heritability and repeatability's were calculated using BLUPF90 family of programs. A single-trait repeatability animal model was used and included a 1-vector phenotype (Brix or Colostrum weight), fixed effects (i.e. calving year, parity, 3-month season of calving, and age at calving), additive genetic variance, random permanent environment effects, and random residual effects. A series of bivariate models were used to calculate genetic correlations of Brix score and colostrum weight with test-day compositional traits. Heritability estimates results for Holstein cow Brix and colostrum weight, were 0.25 and 0.15. Jersey cow heritability estimates were 0.36 and 0.47 respectively. We also observed some significant genetic correlations with Holstein Brix score and test-day milk (-0.23), fat (0.54), and SCS (0.29) having moderate correlations. Holstein colostrum weight had a strong correlation with test-day milk (0.96). Jerseys had strong genetic correlation of Brix score with colostrum weight (-0.98). Low to moderately heritability was observed for Brix score and colostrum weight in both breeds making them receptive to genetic selection in order to improve breeding programs. In conclusion, mode of birth significantly impacted colostrum composition which had subsequent effects on abundance of rumen microbiota. Colostrum Brix and volume were impacted by breed, season, and interaction, and calf incidence of disease was impacted by colostrum composition and environment. Additionally, two factors influencing colostrum quality (Brix score and colostrum weight) were found to be low to moderately heritable and have moderate to strong genetic correlations to compositional traits. Strong significant relationships were also found between colostrum compositional traits and colostrum quality traits. Therefore, incorporating

quality traits into breeding programs has the potential to influence compositional traits which, in turn, can impact calf health and development by the interactions that exist between composition and microbial abundance in the rumen.

GENETIC AND MATERNAL FACTORS UNDERLYING EARLY MILK PRODUCTION AND THEIR INFLUENCE ON CALF HEALTH

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GENERAL ABSTRACT

Factors like cow breed, age, parity, nutrition, environment, and management can affect the quality of early milk produced. Many of these factors have been studied and guidelines developed in order to ensure producers feed the best quality milk to their calves which will allow for calves to develop properly. However, there is still a high death rates in calves younger than 8 wks old. The impact of factors like mode of birth and genetics have not been readily studied. Therefore, two separate studies were conducted looking into impacts of mode of birth and genetics on early milk. The purpose of our first study was to determine mode of birth impacts on composition of early milk and establish relationships between composition and gut bacteria in calves. Charolaise (**CHAR**) and Angus (**ANG**) breeds were used, and early milk samples collected to determine composition. Gut fluid was collected from calves on d 1, 3, 28 after birth and DNA extracted from fluid. Results showed that early milk composition of vaginal delivery (**VD**) cows when compared to cesarean delivery (**CD**) cows were very different. Cows in the VD group were more likely to have increased protein, solids non-fat, and lactose in their milk when compared to CD cows. On the other hand, VD cows had decreased urea concentrations in milk when compared to CD cows. Similarly, short, medium, and long-chain fatty acids were increased in VD cow's milk when compared to early milk of CD cows. These changes in early milk composition between VD and CD cows were associated with a decrease in the calf gut bacteria such as Actinobacteria and Proteobacteria. Results suggest that mode of birth influences protein concentrations in early milk and induces a slight impact on the overall gut bacteria present in the calf rumen microbiome.

A second study was conducted to establish relationships between early milk composition, milk management, and calf health. Additionally, the study looked into determining if factors such as the quantity and quality of milk produced by cows can be passed on from one generation to the next in order to make improvements to milk quality and quantity. Holstein (**HO**) and Jersey (**JE**) cow breeds were used and measurements for milk quantity and quality were recorded from each cow. Additionally, calf records were also obtained to determine relationships between quality and quantity of milk with calf health. Results indicated that early milk quality and quantity were impacted by season and breed; and that calf health was impacted by early milk quantity and quality. Additionally, early milk quality and quantity were found to be heritable, which means these traits can be selected for to make improvements in the quality and quantity of milk in further generations. Finally, results demonstrated that the composition of early milk did not have a relationship with quantity and quality traits, therefore, quality and quantity traits can be selected for without affecting the actual composition of milk.

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LIST OF ABBREVIATIONS

AA - amino acid

ADG - average daily gain

AGIL- Animal Genomic Improvement Laboratory

ANG - angus

BHB – beta hydroxybutyrate

c/ebpδ- enhancer binding protein delta

CD – cesarean delivery

CHAR – Charolaise

CHO – carbohydrate

C3- complement component 3

C4- complement component 4

CP – crude protein

DHIA- Dairy Herd Improvement Association

EGF – epidermal growth factor

FA – fatty acid

FcRN – Fc receptor of the neonate

GLUT1-glucose transporter 1

HO – Holstein

IgA – immunoglobulin A

IGF1 – insulin like growth factor 1

IGFBP-5- insulin like growth factor binding protein 5

IgG – immunoglobulin G

IgM – immunoglobulin M

IL-1 – interleukin 1

IL-6 – interleukin 1

INF – interferon

JE – Jersey

LCFA – long chain fatty acid

MCFA – medium chain fatty acid

MLR – multiple linear regression

NEB – negative energy balance

NRC – National Research Council

PBS – phosphate buffered saline

PIGR – polymeric immunoglobulin receptor

PRL – prolactin

PUFA – polyunsaturated fatty acid

RDP – rumen degradable protein

RUP – rumen undegradable protein

SCFA – short chain fatty acids

SCS- somatic cell score

SFA- saturated fatty acids

SNF – solid non-fat

STAT5 – signal transducer and activator of transcription 5

TFA – total fatty acid

TG – triglycerides

TGF-β1 – transforming growth factor β1

TLR10 – toll like receptor 10

TNF – tumor necrosis factor

TSP – total serum protein

VD – vaginal delivery

VFA – volatile fatty acid

INTRODUCTION

High mortality rate in calves poses a big challenge for dairy industries with approximately 8-11% of calves dying before the weaning period (Jorgensen et al., 2017; Godden, 2019). This can be detrimental to dairy cattle operations with an average annual loss of > $100 million (Lorenz et al., 2011; Cho and Yoon, 2014). The high mortality rates are attributed to diseases such as scours and pneumonia, which are the two deadliest diseases in the dairy industry, with scours accounting for 52.5% of deaths and pneumonia 21.3% (USDA, 2011). Calves are born with less than 10% of immunity needed to protect them against environmental pathogens with total serum protein levels of 4.2 mg/dL (Nocek et al., 1983). Other species such as humans are born immunocompetent because there is placental transfer of immunoglobulins before birth. However, ruminants lack of intrauterine transfer of maternal immunoglobulins across the placenta makes them more susceptible to pathogenic diseases at birth (Besser, 1994). Instead, calves rely on maternal colostrum consumption after birth in order to absorb immunoglobulin G and achieve passive immunity transfer. Success of passive immunity is characterized by > 1000 mg/dL of IgG circulating in calf serum or total serum protein levels > 5.5 mg/dL and can be measured between 24-72 h after first feeding.

For maternal colostrum to promote successful passive immunity transfer, colostrum must be of optimal quality and there must be sufficient colostrum management practices. Colostrum is considered good quality if it has immunoglobulin G (**IgG**) ≥ 5000 mg/dL or 22% Brix value (Merial, 2018). Brix values are determined by the use of a measuring instrument, refractometer, that measures the amount of light refracted while passing through a liquid sample which, in turn, yields a Brix value. The refractometer measures indirect IgG content of colostrum, thus providing farmers with a new, simple, and accessible tool to measure colostrum quality.

Nationwide colostrum management guidelines include feeding 3.78 L of good quality colostrum within the first 6 hours of life without delay before the calf's gut closure and they can no longer absorb immunoglobulins (Godden et al., 2019). Evaluations in the United States have reported that nearly 60% of maternal colostrum is inadequate or of low quality (Morrill et al., 2012). Factors such as dam age, parity, season of calving, nutrition, breed, and dry-period length can all affect colostrum composition which is heavily linked to quality. Genetics of bovine milk have also been studied with various components found to be heritable such as fat, proteins, carbohydrates, and milk volume and incorporated into breeding programs (Heinrichs et al., 2016). Other traits such as IgG content are moderately heritable ($h^2 = 0.41$; Gilbert et al., 1988). Currently, even after improved colostrum management guidelines, selecting for improved milk composition, and improved IgG content of colostrum, mortality rates in pre-weaned calves are still of concern due to the economic impact on the dairy industry. In fact, even after calves have achieved passive immunity transfer (> 1000 mg/dL serum IgG), they are only 4% more likely to survive when compared to calves that did not achieve passive immunity transfer (Sellers, 2001). This suggests that other factors (i.e. maternal and genetic) are involved in the quality of early milk being produced and influence calf incidence of disease. Based on previous literature in mature milk, we hypothesized that colostrum components impact the calf gut ecology and can be used for genetic selection to improve both colostrum quality and volume.

CHAPTER 1: LITERATURE REVIEW

MILK COMPONENT BIOSYNTHESIS

There are multiple components that make up early milk. The major categories of milk components are protein, fat, and lactose. Each of these components has a unique biosynthetic pathway. Additionally, it is now known that milk is also colonized with bacteria that assist in neonatal development. Understanding how each biosynthetic pathway works and the origin of milk bacteria could allow for further manipulation in order to alter the composition of early milk to better fit the nutritional and developmental needs of neonates.

Protein. Milk contains different kinds of proteins that can be divided into two main groups (i.e. specific mammary proteins vs non-specific mammary proteins). Non-specific mammary proteins include immunoglobulins which are one of the most important groups of proteins within early milk. There are 3 classes of immunoglobulins that are functionally important in milk: immunoglobulin G (**IgG**), immunoglobulin A (**IgA**), and immunoglobulin M (**IgM**) (Stelwagen et al., 2008). Each immunoglobulin has its own function. Immunoglobulin G is important for providing passive immunity to offspring. Immunoglobulin A helps protect mucous membranes in intestines which is important for microbial development. Additionally, IgM is the first immunoglobulin produced as the first line of defense against pathogens (Hurley and Theil, 2011). Immunoglobulins are transferred from the dam's bloodstream into the mammary gland through receptor complexes during the process of colostrogenesis. These receptor complexes are Fc receptor of the neonate (**FcRN**) and polymeric immunoglobulin receptor (**pIgR**). Each receptor is responsible for the mass transfer of specific types of immunoglobulins with FcRn transferring IgG and pIgR transferring IgA and IgM (Trurula and Wobus, 2018; Baumrucker and Bruckmaier, 2014). Immunoglobulins are mainly present in colostrum and their concentrations decline rapidly as lactation advances. This is thought to be due to down regulation of genes that

were present during colostrum and this leads to cessation of influx of immunoglobulins and a dilution effect due to production of other milk components such as lactose (Baumrucker and Bruckmaier, 2014).

Next, there are milk specific proteins such as caseins (α-casein, β-casein, γ-casein, and κ-casein) and whey proteins (α-lactalbumin, β-lactoglobulin, lactoferrin), whose synthesis is affected by the feed proteins provided in the diet. There are two main categories of feed proteins in ruminants: rumen undegradable protein (**RUP**) and rumen degradable protein (**RDP**) with each category having their own pathway to becoming milk proteins. Rumen undegradable proteins pass through the rumen unchanged and reach the abomasum where mammalian secretions such as pepsin carry out the initial protein degradation into amino acids. The amino acids are then absorbed into the small intestine by Na^+- dependent amino acid transporters, which bind amino acids only after binding sodium and undergoing a conformational change (Bowen, 2019; Kellems et al., 2002; Moran, 2005). Rumen degradable protein is digested in the rumen where microbes metabolize the protein into ammonia. The ammonia is then utilized by the microbes in order to synthesize microbial proteins which continue to the abomasum and subsequently the small intestine and undergo the same process as RUP (Kellems et al., 2002). Now there are amino acids available for synthesis of milk proteins; however, they must first reach the mammary gland. After the small intestine absorbs amino acids, they exit through the flow of oxygen-poor blood in the small intestinal villi and enter venous circulation through the hepatic portal vein and later into arterial circulation (Bolton and Wright, 1936). Once in arterial circulation, the amino acids are transported into the mammary gland with the aid of enzyme gamma glutamyl transpeptidase and a Na^+-independent transporters transporter (Shennan et al.,1997).

Once in the mammary gland, the amino acids are used to create milk proteins. Milk protein synthesis follows the typical pattern described in other mammalian species (Akers., 2002). Milk proteins are only made during lactation due to gene and hormonal regulation. In the mammary gland, genes such as *Signal transducer and activator of transcription 5* (***STAT5***) regulate mRNA expression which in turn dictates the production of milk proteins such as whey and casein. Additionally, lactogenic hormones such as prolactin, which have increased production during lactation, exert their effect after parturition and promote transcription of milk protein mRNA. Messenger RNA leaves the nucleus and reaches the cytoplasm and binds to a small ribosomal subunit. After, tRNA transfers amino acids to the ribosome where a growing peptide chain starts to develop by linking amino acids that are continually being transferred by tRNA. Once the growing chain reaches a stop codon it is released from the ribosome and a milk protein is formed (Akers, 2002).

Fat. Fat is one of the most variable components in milk. It is primarily composed of triglycerides (**TG**) which are made up of 3 fatty acids (**FA**) and a glycerol backbone. There are three main sources of FA in milk TG: 1) conversion of glucose to pyruvate, citrate, and acetyl CoA (non-ruminants), 2) diet, and 3) *de novo* fatty acid synthesis. The conversion of glucose to pyruvate as a FA source occurs in non-ruminants, therefore, only FA sources from diet and de novo fatty acid synthesis will be discussed. From the diet, fatty acids are greater than 14 carbons (palmitic, stearic, oleic, or linoleic) while shorter chained fatty acids are derived from *de novo* fatty acid synthesis (Akers, 2002).

De novo fatty acid synthesis, which forms short and medium chain fatty acids (**SCFA** and **MCFA**), accounts for 50% of milk fat and uses 2 main precursors that originate from the cow's blood: beta-hydroxy butyrate (**BHB**) and acetate. Both BHB and acetate are end products of

rumen microbial fermentation. Acetate is the major de novo FA synthesis precursor in cattle; it travels from the blood and diffuses through capillaries where it can then enter mammary epithelial cells. Once in the mammary epithelial cells, fatty acids are synthesized in the cytoplasm. The acetate molecule is converted into acetyl CoA which is then acted upon by the enzyme acetyl CoA carboxylase and becomes malonyl CoA. It then enters the malonyl CoA pathway which through the aid of fatty acid synthase allows for the addition of 2 carbon units at a time to the growing fatty acid chain (Akers, 2002).

The remaining 50% of milk fats come from the diet from preformed dietary lipids (i.e. TG) and phospholipid membranes of forage components. In the rumen, microbes use a phospholipase enzyme to hydrolyze TG and forage phospholipids into glycerol and polyunsaturated fatty acids (**PUFA**). The microbes then perform biohydrogenation, addition of H atoms, of fatty acids to convert them to saturated fatty acids (**SFA**) that are also long chain fatty acids (**LCFA**). From here, the glycerol is fermented to one propionate molecule and the fatty acids are transported to the small intestine for absorption. Once in the jejunum of the small intestine glycerol and fatty acids fuse back together and then proteins are added to form a lipoprotein, which facilitates the transport of lipids (Daniels, 2019). The lipoprotein then forms vesicles called chylomicrons that are released from epithelial cells of the jejunum through exocytosis and released into the lymphatic system and then into arterial circulation. The milk TG precursors then enter the mammary cell by diffusing through the basolateral membrane and undergo assembly near the smooth and rough endoplasmic reticulum. After assembly, milk fat droplets travel to the membrane, bind, and then exit the cell (Metka and Nada, 1992). As with milk protein biosynthesis de novo fatty acid synthesis only occurs during lactation. The lactogenic hormone prolactin is in increased concentration during lactation which facilitates the regulation of

mammary epithelial cell specific lipogenic gene expression thus controlling de novo fatty acid synthesis.

Carbohydrate. Lactose is the most common carbohydrate within milk (Akers, 2002) and synthesis only occurs during lactation due to it being hormonally regulated. Lactose is a disaccharide composed of one molecule of glucose and one molecule of galactose. The biosynthesis of lactose starts in the rumen in which dietary carbohydrates are fermented by microbes into propionate. Propionate is then absorbed through the rumen papillae and into the bloodstream where it travels to the liver and undergoes gluconeogenesis (Kleiber et al., 1952). The process of gluconeogenesis produces glucose which exits the liver through the hepatic vein and travels to the mammary gland epithelial tissue. Once here, glucose transporter 1 (**GLUT1**) allows for the transport of glucose into the mammary gland. Glucose then undergoes a series of catalytic events with the most important one being the conversion of glucose to galactose by UDP-galactose-4-epimerase, which changes the orientation of an -OH at the 4^{th} carbon on glucose. After the conformational change of glucose to galactose, lactose precursors move into the Golgi apparatus. In the Golgi, the enzyme galactosyl transferase adds galactose to glucose to form lactose in the presence of α-lactalbumin (Akers, 2002). Once lactose is formed, it travels from Golgi in secretory vesicles to the membrane where it uses merocrine mode of secretion, which means its excreted through secretory cells via exocytosis but does not pinch of an apical portion of cell like in apocrine excretion (Kleiber et al., 1952).

Early milk microbes. Previously it was believed that early milk was sterile, however, recent studies have shown that it contains varying amounts of commensal, mutualistic, and potentially probiotic bacteria that assist in the development of a healthy neonate (Fernandez et al., 2012). Scientists have developed the entero-mammary pathway theory which provides a

potential explanation for the origin of microbes within milk (Rainard, 2017). The entero-mammary pathway originated from evidence that suggests that immune cells in the gut lamina propria can migrate to the mammary gland and into milk during lactation. Fernandez et al. (2012) showed that dendritic cells can retain live commensal bacteria in mesenteric lymph nodes which can spread to other locations through circulation. These antigen-stimulated cells are attracted to colonize other distant mucosal surfaces such as the mammary gland. However, this pathway is more readily accepted in humans rather than ruminants due to lack of migration of mesenteric lymph node cells and the mammary gland not being considered a common part of the mucosal immune system (Rainard, 2017). One possible explanation for colonization of microbes in the mammary gland is the passage of dead or circulating bacteria from the blood to milk carried inside of phagocytic cells. These cells would cross the epithelium of the mammary gland and be shed in milk (Rainard, 2017).

Culture-dependent and independent techniques have revealed the dominance of staphylococci, streptococci, lactic acid bacteria, and Bifidobacterium in early milk (Fernandez et al., 2012). However, early milk composition changes depending on stage of lactation. Studies by Cabrera-Rubio et al. (2012) show that colostrum was primarily compromised of *Weisella* and *Leuconostoc,* which are two lactic acid bacteria, as well as *staphylococcus* and *streptococcus*. Once the animal was further into lactation at 1 and 6-month post-partum, they still had lactic acid bacteria among the most abundant with the addition of *Veillonella, Leptotrichia,* and *Prevotella*. Cabrera and colleagues also observed different patterns of bacterial diversity with colostrum having greater diversity than transition and mature milk.

EARLY MILK PRODUCTION

The average lactation period for a dairy cow is 305 days, after which, pregnant animals undergo a dry period of 60-days on average. The dry period is marked by extensive changes to the mammary gland tissue and biosynthetic processes that allow for influx of nutrients and immune components into colostrum. Involution is the first process to occur during the dry period. During involution, mammary gland tissue goes through a complex multi-phase process where the previously lactating gland returns to its pre-pregnancy morphological state (Stein et al., 2007). The first phase of involution is marked by apoptotic events that are regulated by transcription factors such as STAT3 which signals genes such as insulin-like growth factor binding-protein 5 (***IGFBP-5)*** and enhancer binding protein delta (**c/ebpδ**) to trigger apoptosis. At this stage, apoptotic cells can then be seen in the lumen of mammary alveoli because of detachment of cells from alveolar structure and shedding into lumen (Watson, 2006). After active involution, the mammary gland enters a steady state where the gland is in a non-lactating state and is not undergoing changes (Hurley, 1989). Next, involution of the mammary gland enters the second phase which is marked by collapsing alveoli and refilling of adipocytes regulated by serine proteases that break down the extra-cellular matrix (Watson, 2006).This allows for tissue remodeling and prepares the mammary tissue for the final stage of involution which is the redevelopment. During redevelopment, mammary tissue becomes differentiated with apoptosis ceasing, stroma of mammary gland become less evident, and alveoli cells becoming secretory once again leading into lactogenesis (Stein et al., 2007).

There are 3 major stages of lactation: 1) colostrum production, 2) transition milk production, and 3) mature milk production. The milk produced at each of these stages differ in composition and have specific roles to play for calf health and development. Therefore, comprehending the major

differences of the major lactation stages is important to determine impacts to a growing and developing neonate.

Colostrum. Lactogenesis is the onset of milk production and is divided into two stages (i.e. lactogenesis I and II; Pillay and Davis, 2019; Tucker, 1980). During lactogenesis I, the placenta supplies high levels of progesterone which inhibits further mammary cell differentiation and allows for the initiation of colostrum production. The process by which colostrum is produced is referred to as colostrogenesis and occurs as part of lactogenesis I. Colostrogenesis is initiated approximately 5-6 wks pre-partum and is marked by the influx of nutrients and immunoglobulins into the mammary gland (Hill, 2010; Quesnel and Farmer, 2018). Immunoglobulins are transported from the blood stream and into mammary gland through the Fc receptor of the neonate (**FcRN**; IgG) and polymeric immunoglobulin receptor (**pIgR**; IgA and IgM) via transcytosis (Baumrucker and Bruckmaier, 2014). Nutrients (i.e. milk specific and non-specific proteins, lactose, fats) each have their individual biosynthetic pathways via which they enter the mammary gland which will be discussed more in depth further on.

The end of colostrogenesis is marked by hormonal changes such as a decrease in progesterone levels and increased levels of cortisol and prolactin which initiate lactogenesis II (Pillay and Davis, 2019). This occurs approximately 0-4 days pre-partum and allows for the release of colostrum in order to provide nutrients for the neonate (Barrington et al., 2001). The increase in prolactin at birth also signals alveolar cells in the mammary gland to cease expressing immunoglobulin receptors which stops the influx into colostrum (Godden, 2019). Once the cow starts lactation, colostrum will be produced for the first 24 hours post-partum and during this time there will be compositional changes in preparation for transition milk production.

Initial composition of bovine colostrum is very rich in immunoglobulins (3000-6000 mg/dL), high in protein (6-13%), low in carbohydrate (1-2%), and high in fat (4-8%) within the first 24 hrs post-partum (McGrath et al., 2016). Colostrum also contains other components such as growth factors, enzymes, and cytokines. Growth factors in colostrum include epidermal growth factor (**EGF**; 324 μg.L−1), insulin like growth factor 1 (**IGF-1**; 50 to 2000 μg.L−1), transforming growth factor-β1 (**TGF-β1**; 12 to 43 μg.L−1), and lactoferrin (0-12 mg/ml). The role of these growth factors is to stimulate cell growth and promote healthy gut development, renal development, and liver development (Pakkanen and Aalto, 1997; Gauthier et al., 2006).The concentration of these growth factors is highest immediately after parturition and decrease quickly over time (McGrath et al., 2016). Colostrum also has enzymes such as proteinases, lipases, and esterases which are high after parturition and either remain stable through lactation or decrease rapidly after birth. The enzymes can act as bacterial barriers or allow for proper degradation of macromolecules when consumed by the neonate (McGrath et al., 2016). There are also cytokines, such as interleukins (**IL-6, IL-1 β**; 77±31 ng/ml, 844±299 ng/ml), tumor necrosis factors (**TNF-α**; 926±16 ng/ml), and interferons (**INF-γ**; 261±59 ng/ml), in colostrum and the concentrations of these are highest in colostrum when compared to any other of the early milk lactation stages (Hagiwara et al., 2000). While considered to be in minute concentrations they still have significant biological effects with the primary role being modulation of the immune system (McGrath et al., 2016). They stimulate maturation of the immune system in calves by enhancing mitogenic responses and eliciting interleukin mRNA and CD25 expression in peripheral blood mononuclear cells (Yamanaka et al., 2003). As a whole, the composition of colostrum allows for the development of the metabolic system by establishing endogenous glucose production and increasing glucose absorption, immune function by promoting

antimicrobial effects in host-defense against microbes, and intestinal mucosal layer by promoting better absorption of nutrients and increased villi growth (McGrath et al., 2016).

Colostrum components undergo steady compositional changes within the first 24 hours post-partum. These changes are a decrease in protein, fat, and an increase in carbohydrate which mark the start of the second stage of early milk production, transition milk.

Transition milk. At 25 to 72 hrs post-partum, colostrum begins to shift to the second stage of lactation, transition milk. The composition of transition milk is unique in the sense that it is still similar to colostrum but also has compositional properties of mature milk.

The composition of transition milk differs from colostrum: decreased levels of protein (6-8%), increased lactose (3-4%), and decreased fat (4-5%; McGrath et al., 2016). This increase in milk lactose being produced supports the nutritional and developmental needs of the growing neonate by enhancing the intestinal absorption of calcium and this, in turn, promotes healthy bone growth for the calf (Ballard and Morrow, 2013). Additionally, lactose promotes the growth of beneficial intestinal microflora (McGrath et al., 2016).

Since transition milk still has some compositional properties of colostrum, it can also continue to supply moderate amounts of different protein groups (i.e. specific vs non-specific mammary proteins) and immunological components like cytokines and immunoglobulins. Lactoferrin is a considered a growth factor within the whey proteins with antibacterial, antimicrobial and antifungal properties that bolsters immunity and assist in the development of healthy gut microbiota (Sovereign Laboratories, 2017). Growth factors like lactoferrin will assist in proliferation of different cell types in order to promote proper gut, renal development, and liver development (Agriland, 2019; Hanson, 2019). In the gut, they promote decreased crypt fission of the small intestine as well as crypt hyperplasia, whereas in the kidneys, liver, and lungs

it promotes proper development of blood vessels (angiogenesis), tissue, and hematopoiesis (Gauthier et al., 2006). The concentration of lactoferrin decreases gradually over stages of lactation with an average 2.0 mg/ml in transition milk (Yang et al., 2018). Additionally, alpha-lactalbumin, another whey protein which plays an important part in milk secretion, is found in transition milk at an average 2.0 mg/ml which is about the same as the concentration in colostrum (Pereze et al.,1989). Immunoglobulin levels, which provided passive immunity to calves at birth, decrease and are now at 2000 mg/dL (Godden, 2019). Furthermore, cytokines also contribute to immune function by promoting higher percentage of lymphocytes in the blood indicating progressive maturation of the adaptive immune response (Novo et al., 2017). There are steady changes occurring during transition milk production. These changes in composition mark the start of the third stage of early milk production, mature milk.

Mature Milk. Milk produced after 72 hours post-partum is considered mature. On average, mature cow milk is 87.7% water, 4.9% lactose (carbohydrate), 3.4% fat, 3.3% protein, EGF (155 μg.L−1), IGF-1 (<10 μg.L−1), TGF-β1 (0.8 to 3.5 μg.L−1), IL-6 (0.22 ng/mL), IL-1 β (3.43 ng/mL), TNF-α (4.58 ng/nL; Ballard and Morrow, 2013; Hagiwara et al., 2000). Though 80% of total protein in colostrum consists of immunoglobulins, there are little to no immunoglobulins in mature milk which contribute to the decrease in protein (500 mg/dL; McGrath et al., 2016). Additionally, growth factors, like lactoferrin are in minute amounts at 1.0 mg/ml, while alpha-lactalbumin has seen a gradual decrease over lactation stages and on average is at 1.5 mg/ml in mature milk (Perez et al., 1989; Yang et al 2018). Mature milk composition is also mainly water, which is due to the gradual increase of lactose throughout the stages of lactation. Lactose is the major osmole in milk, which accounts for 50% of milk's osmotic

pressure, therefore, as lactose concentration increases to meet the growth demands of the neonate so does the milk volume.

Cytokine levels have also significantly decreased in mature milk with some cytokines becoming undetectable. The decline observed in growth factors and cytokines, as well as increase in lactose is due to changes in the needs of the developing neonate. At this stage neonates have received the necessary components to develop a healthy immune system and mature milk is reared towards nutrition and maintenance. It also provides the energy for proper growth and development. Aside from components like protein, lactose, and fat; milk also contains micronutrients such as vitamins and minerals. These vitamins and minerals help with immune function, metabolic regulation, and development of bone, teeth, muscle and eyesight (Dimbylow, 2019).

FACTORS AFFECTING MILK QUALITY

Many factors can influence the biosynthetic process of milk production: maternal, environmental or genetic factors. Disruptions to these biosynthetic pathways can cause changes to early milk composition with subsequent detrimental effects to milk quality.

Dam Age and Parity. Most studies report that older cows tend to produce higher quality colostrum because older animals have had an increased exposure to pathogens through increased parities, which results in increased antibody production (Godden, 2019). Older cows that have had 3 or more lactations produced 19.5 g /L more IgG in their colostrum than first lactation cows. Colostrum of first-calf heifers contained 5.68% total immunoglobulin, whereas third and fourth parity cows had 7.91% and 7.53% total immunoglobulin, respectively. There was a significant difference in IgA and IgG content of first parity when compared to third and fourth parity cows, whereas after the third parity no significant differences in colostrum immunoglobulin concentration was found. First-calf heifers had significantly less IgG (4.68 %)

and IgA (0.76%) in their colostrum when compared to third (IgG = 6.04%; IgA = 1.65%) and fourth (IgG = 5.61%; IgA = 1.40%) parity cows (Muller and Ellinger, 1981). Additionally, Tyler et al (1999) reported similar findings in Holstein cows, but reported that there was no significant differences in IgG concentration of Guernsey cows colostrum among first (119 g/L), second (113 g/L) and ≥ 3 (115 g/L) lactations.

Studies have also shown that parity affects protein concentrations in colostrum as well. Significant differences were found in total protein concentration of first-calf heifers when compared to third and fourth lactation cows. Results showed third and fourth lactation cows had total protein concentrations of 15.1 g/dL and 16.4 g/dL, whereas first-calf heifers had 14.5 g/dL (Rocha et al., 2014). Additionally, increased levels of milk specific proteins such as α-lactalbumin, which plays an important role in certain colostrum component biosynthesis, were observed. Results were similar to total protein concentrations with third (354 mg/dL) and fourth (418 mg/dL) lactation cows having greater concentration of α-lactalbumin when compared to first-calf heifers (241 mg/dL; Rocha et al., 2014). Overall, there is disagreement in literature on the impacts of dam age and parity on colostrum quality. Therefore, automatically discarding colostrum of first-time heifers should be discouraged and instead on farm management practices should focus on testing their colostrum quality.

Dam Breed. The quality of early milk can be affected by the animal's breed. Each breed, while physiological processes are essentially the same, show varying degrees of IgG concentration. Immunoglobulin G concentrations were greater in colostrum of beef cows at a concentration of 113.4 mg/L, whereas dairy cows had decreased IgG concentrations in early milk at 42.7 mg/L (Muller and Ellinger, 1980). Results showed that total immunoglobulin concentration in different dairy cow breeds averaged 8.1% for Ayrshire, 6.6% for Brown Swiss,

6.3% Guernsey, 5.6% for Holstein, and 9% for Jerseys (Guy et al., 1994). Jerseys had the highest IgG, IgA, and IgM concentration among the 5 dairy breeds while Holsteins had the lowest concentration of IgG and Guernsey's had the lower concentration of IgA and IgM among the dairy breeds studied. The differences observed in early milk quality due to breed could also be attributed to sire lines (Godden, 2019). Hereford × Angus crosses had increased IgM and IgG in early milk when compared to purebred Hereford cows. There were differences in IgM yield in colostrum of dams that had been mated to a specific breed of bull. For example, dams that were mated to Simmental bulls had an overall lower concentration of IgM when compared to dams that were mated to bulls of other breeds, but no change was observed for IgG (Norman, 1981). This suggests that differences in breed does affect quality of early milk produced (Muller and Ellinger, 1980).

Dam Genetics. Early milk quality traits have been studied and genetic correlation have been determined between quality traits (i.e. volume and Brix) and composition traits (i.e. lactose, protein). In Holstein cows, strong negative genetic correlations were observed between lactose with protein content (r_g = -0.96) and Brix score (r_g = -0.89). The strong genetic correlation means both of these traits are regulated by overlapping genes which means that selection for increased quality (i.e. Brix, protein content…) will heavily impact lactose content by decreasing it. Lactose synthesis is needed to maintain osmotic equilibrium; therefore, decreased lactose synthesis will decrease water secretion and consequently early milk yield to an extent (NRC, 1988). Additionally, a strong positive genetic correlation also existed between Brix and protein (r_g = 0.97; Soufleri et al., 2019). This means selection for increased quality (Brix) will heavily impact the protein content of early milk. Brix is an indirect measurement of colostrum quality and

represents the protein density within early milk, therefore, the observed relationship between Brix and protein is expected.

Heritability for early milk quality traits and compositional traits have also been studied. Colostrum yield for Holsteins was lowly heritable (h^2 = 0.04), whereas milk yield heritability was much higher (h^2 = 0.54; Loker et al., 2009). Additionally, Brix score was moderately heritable (h^2 = 0.27; Soufleri et al., 2019) and there are no other references in the literature for Brix score heritability. Soufleri and colleagues also reported colostrum fat protein, and lactose to be moderately heritable (h^2 = 0.21, 0.19, and 0.15, respectively). Milk fat, protein, and lactose had much higher heritability values with h^2 = 0.56, 0.58, and 0.48 when compared to colostrum compositional traits (Loker et al. 2009). Discrepancies in these values could be attributed to differences in the amount of data recorded for both milk and colostrum traits. Additionally, Jerseys have no known reported genetic parameters for colostrum quality traits, but milk yield heritability estimates were similar to Holstein cows (h^2 = 0.37). Milk traits for Jersey have reported heritability values for fat, protein, and lactose as h^2 = 0.42, 0.44, and 0.20, respectively (Missanjo et al., 2013; Roveglia et al., 2017). Based on these findings, we suggest that genetic improvement of early milk is possible through genetic selection with traits having higher heritability and stronger genetic correlations possibly providing faster improvements within a heard.

Dam Nutrition. Preparturient cows require a specific diet that will meet all their metabolic, gestational, and maintenance needs. This diet includes a balance of crude protein (**CP;** of which 16% is nitrogen), phosphorus (0.30-0.40% of dry matter), and potassium (1-2% of dry matter) as recommended by the National Research Council (**NRC**). The diet fed to the dam can impact composition of early milk and, in turn, impact quality. Immunoglobulin content,

which is a measure of quality of early milk, was impacted by pre-partum diet of cows fed high energy (0.69 UFL/kg DM, NDF 52% DM versus low energy (0.61 UFL/1kg DM, NDF 56% DM) diets (Hough et al.,1990). Cows fed low energy diets had increased IgA content of early milk compared to high energy cows (Nowak et al., 2012). Bain (2020) proposed that diets not supplying appropriate levels of energy content can significantly reduce immune function. This exposes the animal to greater risk for infections which is potentially why an in increase in IgA was observed in animals fed lower energy diets. Additionally, nicotinic acid supplementation (48 g/d) during the dry period was also observed to increase IgG concentration in colostrum from 73.8 to 86.8 g/L (Aragona et al., 2015). Cows fed a total mixed ration (TMR) diet formulated to supply 125-150% of energy recommended by the NRC had increased yield of de novo fatty acids and insulin levels but decreased levels of IgG. However, when fed the standard diet recommended by NRC cows had normal levels of colostrum IgG but not nearly the same concentration of fatty acids or insulin (Mann et al., 2016). Other components such as early milk fat and protein can also be affected by diet (NRC, 1988). Fat percentage in milk can be altered by dietary factors that affect rumen fermentation. One example is decreasing the forage to concentrate ratio in the diet which affects rumen fermentation by lowering the pH. The low pH in the rumen increases propionic acid production and reduces fiber digestion which declines fat percentage of early milk proportionately (NRC, 1988). The changes in milk fat percentage with the use of fat in diets can be attributed to changes in output of different fatty acids from the mammary gland (i.e. SCFA, LCFA, MCFA; NRC, 1988). Early milk protein can be affected by dietary crude protein concentrations; a 1 unit increase in dietary crude protein between 9-17% was associated with a 0.02% increase in early milk. Additionally, different sources of dietary crude protein can also impact early milk protein content. Cows fed high rumen degradable

protein had elevated early milk protein most likely from milk urea. However, feeding low rumen degradable protein increases amino acid supply for the mammary gland and more true protein is produced (NRC, 1988).

Based on previous research, diet appears to impact early milk composition, therefore affecting quality. Impacts to colostrum quality are seen more prominently when diets deviate from the NRC guidelines. The impact on early milk can vary depending on what supplements are implemented into the diet and how TMR is formulated. This means diets can be tailored towards producers' goals and it is possible to manipulate early milk composition. However, because of the variation that exists from one study to the next much more research is needed to determine what diet is truly the best for cows to yield early milk of optimal quality.

Early Milk Volume. The volume of colostrum produced by the dam after birth is considered an important factor in determining quality. Previous research by Pritchett et al. (1990) and Silva del Rio et al. (2017) investigated sources of variation in IgG concentration of early milk. The volume of colostrum produced by the dam had a negative moderate correlation with IgG concentration (r = -0 .29; r = -0.37). Pritchett and colleagues observed that cows producing less than 41.60 kg had increased quality colostrum, defined as colostrum with > 50 g/dL of IgG, than high volume producing cows. An increase in IgG concentration by 97.7 g/L was observed in low colostrum producing cows while high colostrum producing cows had a mean IgG concentration of 71.6 g/L (Silva del Rio et al., 2017). In these studies, a negative relationship between quality and yield is observed. The authors suggested a dilution effect where the more volume of early milk diluted the concentration of IgG. Additionally, lactose varies in concentration during early milk production stages. For example, during colostrum production there is an average of 2.7% lactose, subsequently, transition and mature milk have lactose

concentrations of 3.9% and 5% (Godden et al., 2019). As lactose production increases during the different stages of milk production, there is an increase in volume of early milk production because of lactose role as the major osmole in milk. Lactose is important during these stages of early milk production and can be considered a component that contributes to quality. It promotes intestinal absorption of calcium, healthy bone growth for the calf, and beneficial intestinal microflora (McGrath et al., 2016; Ballard and Morrow, 2013). Proteins, such as the whey protein lactoferrin, can also contribute to early milk quality by bolstering immunity and assisting in the development of healthy gut microbiota (Sovereign Laboratories, 2017). There is an inverse genetic correlation between protein content and volume (r_g = -0.45; Petrini et al., 2016). Average lactoferrin concentration decreases from colostrum to mature milk: 3.0 mg/mL in colostrum, 2.0 mg/mL in transition milk, and 0.5 mg/mL in mature milk, whereas volume of milk produced increases as it becomes mature milk (Yang et al., 2018). Therefore, early milk volume can impact the amount of protein in early milk produced and have impacts on quality.

Season of calving. The season when a cow calves can influence the quality of the milk being produced via heat stress or changes in daylength. Increased specific gravity and protein levels were present in colostrum during autumn months for Holstein and Jersey cows, and the lowest readings for specific gravity and protein were in the summer for Ayrshire and Brown Swiss cows (Morin et al., 2001). Cows exposed to heat stress in late gestation yielded decreased mean concentrations of IgG and IgA, decreased percentages of total protein including casein, lactalbumin, fat, and lactose, decreased short- and medium-chain fatty acids, and decreased energy in early milk (Nardone et al., 1997). Collectively, these findings suggest that changes in composition may be attributed to the negative effects of heat stress. Heat stress may cause nutritional restriction, decreased peripheral blood flow, and impaired immune reactivity with

subsequent effects on transfer of IgG and nutrients from the blood to the udder. This can be observed in a study conducted on heat stressed dairy goats by Sano et al. (1985). They observed lower influx of nutrients such as glucose into the mammary gland accompanied by decrease plasma flow. This ultimately decreased lactose concentrations and immunoglobulin concentration. They also observed a decrease in dry matter intake which also impacts the body's ability to utilize nutrients for biosynthetic processes.

Mode of Birth and Hormones. Whether a dam delivers vaginally or through cesarean section can affect the quality of colostrum being produced. While cesarean section in cattle is uncommon, research in humans can help to better comprehend the differences observed in early milk composition in dams who delivered vaginally or through cesarean. The compositional differences observed have mainly been attributed to disruptions in hormonal regulation (Dizdar et al., 2014) and subsequent effects to transcription factors and gene regulation. One example is Prolactin (**PRL**) concentrations in women that delivered through cesarean section (**CD**) or vaginally (**VD**) were significantly increased for VD compared to CS at the onset of lactation (Nissen et al., 1996). Prolactin concentrations may have as a feedback mechanism that either downregulated or upregulated transcription factor STAT5. This has subsequent effects on regulation of milk protein gene expression which will either promote or demote protein synthesis (Osorio et al., 2016).

Women who delivered via cesarean section have decreased protein levels which downregulates *B4galt1* and lactose concentration (Osorio et al., 2016). The biosynthetic process for lactose relies heavily on protein availability. Specifically, it needs alpha lactalbumin as a signal to convert glucose to galactose. The gene *B4galt1* was regulated by the presence of this protein and encodes for the enzyme needed to convert glucose to galactose. In addition, the

process of fatty acid synthesis can also be affected by the change in PRL concentration due to mode of birth. It hinders the integration of acetate into fatty acid biosynthesis via dietary fibers and decreases synthesis of lipids which in turn affects fatty acid synthesis via dietary fats (Waters and Rillema, 1988).

Additionally, microbial differences have been observed among women that delivered vaginally versus cesarean section. Colostrum from mothers that delivered vaginally had greater biodiversity when compared to cesarean section colostrum. Furthermore, colostrum of cesarean section mothers was richer in microbes belonging to *Streptococcus* and *Haemophilus* genera (i.e. Firmicutes and Proteobacteria phyla). Colostrum of vaginal delivery mothers was richer in microbes belonging to genera *Finelgoldia*, *Halomonas*, *Provetella*, *Staphylococcus*, and *Pseudomonas* (i.e. Firmicute, Proteobacteria, and Bacteroidetes phyla; Toscano et al., 2017). Bacterial phyla such as Firmicutes, Proteobacteria, and Bacteridetes all play a role in the gut and immune development of calves which makes them an important component for early milk quality (Malmuthuge et al., 2012; Malmuthuge et al., 2015).

EFFECTS ON PRE-WEANED CALF

Early milk quality can also impact the pre-weaned calf in multiple aspects. Some of the impacts can be observed to gut development, immune performance, calf performance, and survivability which will be discussed more closely. Comprehending how quality impacts the pre-weaned calf is beneficial in order to promote healthy gut development, enhance immune performance, improve performance traits, and decrease calf mortality.

Calf Gut Development. Calf gut development is a long process that begins shortly after birth. The goal is for the calf to develop a healthy gut by providing the appropriate diet, environment, and optimal early milk quality to decrease the incidence of enteric infections

(Malmathuge et al., 2015). Calf gut development can be affected by factors such as gut microbiome and quality of early milk ingested.

The neonatal gut is colonized by microbes during and after birth (Malmuthuge et al., 2015). The early gut microbiome consists of bacterial phyla such as Bifidobacterium, Firmicutes, Actinobacteria, and Bacteroidetes (Malmathuge et al., 2015). Abe et al. (1995) suggested that increased abundance of Bifidobacterium decreased the prevalence of E coli. and incidence of diarrhea. They also indicated that increased abundance of Firmicutes improved feed conversion and weight gain. This means that a relationship exists between the microbiome with calf health and performance. Continuous exposure to host-specific microbes and maintaining "healthy" bacterial densities is essential for healthy gut development (Malmuthuge et al., 2015; Malmathuge and Guan, 2017). When "normal" bacterial densities are not present it can result in dysbiosis that can lead to susceptibility to a number of pathogenic diseases (Oikonomou et al., 2013). Understanding the relationship between the milk microbiome and gut development could provide strategies to improve the gut health of calves; thereby, improve calf health and performance.

Gut development can also be influenced by the quality of the early milk ingested. Yang et al. (2015) observed increased absorption rates of immunoglobulins, longer intestinal villi, larger villi width, larger crypt depth, larger villi to crypt ration, and more mucosal thickness in calves fed excellent quality colostrum when compared to calves fed low quality colostrum. Overall, the structure of the gut was well developed with uniform villi and deep crypts for calves given better quality early milk. Hammon and Blum (1997) found a greater capacity for absorption in calves fed colostrum versus those that were only fed milk replacer. This shows that colostrum is a

crucial part in the development of a healthy calf gut and that quality of the colostrum fed can significantly impact structural soundness and function.

Immune Performance. Early milk immunoglobulin composition, specifically IgG, can impact the immune performance of neonatal calves by enhancing host-defense mechanisms (Yang et al., 2015) through achievement of passive immunity. When considering proper immune performance in a calf one must consider the development of the complement system. Additionally, colostrum management practices and the gut microbiome can also impact the immune development of a calf. First, the complement system plays a role in passive immunity and provides antimicrobial effects in host-defense against microbes until the calf's own immune system matures (Korhonen et al., 2000). It also involved in both specific and non-specific immunity. Approximately 20 proteins are involved in the complement system with complement components 3 and 4 (**C3; C4)** playing the most important roles. Adequate content of C3 and C4 protein must be maintained in order to avoid reduction of defense mechanisms. Immunoglobulin G in early milk significantly improves the complement systems function by increasing complement protein levels in calf serum. Yang et al. (2015) confirmed this by demonstrating that calves fed milk with proper levels of IgG (50 g/L) increased complement proteins by 2 d after birth in calf serum and stabilized by d 7 compared to calves fed with decreased levels of IgG. Interestingly, there was a spike in compliment proteins in calves fed milk with decreased IgG, which was a result of acute inflammation due to illness (i.e. diarrhea and hemafecia).

Colostrum management includes quality of colostrum fed to the calf, timing of feeding, and amount consumed. National guidelines recommend calves consume 4 Q of colostrum that has a Brix score of 22% or greater within 0-6 h post-partum (IgG > 50 g/mL; Godden et al., 2019). Calves fed maternal colostrum with a Brix of 22% or greater are associated with total

serum protein levels of 5.5 g/dl and is considered success of passive immunity transfer. Most of the proteins in calf serum within the first week post-partum came from maternal colostrum, therefore, total serum protein is a good measure of IgG content and success of passive immunity (Cullens, 2017, Godden et al., 2019). Calves fed 4 Q of colostrum at 0 h post-partum and another 2 Q at 12 h had mean IgG level 31.1 g/L. Conversely, calves fed 2 Q of high-quality colostrum at 0 hours post-partum and a further 2 Q at 12 hours had mean IgG 23.5 g/L (Godden et al., 2019). Additionally, calves fed colostrum 45 min after birth had higher efficiency of absorption and maximum serum IgG levels (IgG 25.5 g/L). Calves fed at 6 h or 12 h post-partum (IgG 18.2 g/L; IgG 5 18.5 g/L, respectively) had significantly less ($P < 0.05$) serum IgG levels. The gut microbiota was also affected by timing of feeding with earlier feeding resulting in more rapid bacterial colonization with organisms such as Bifidobacterium spp (genus) which is part of the phyla Actinobacteria (Godden et al., 2019). Actinobacteria is associated with immune cells and are thought to modulate specific pathways involving innate and adaptive immune processes in the gut (Ruiz et al., 2017). Additionally, other bacterial species in the gut have also been associated with immune development. Lactic acid bacteria, which pertains to the phyla Firmicutes, have been associated with increased Toll Like Receptor 10 expression (**TLR10**) suggesting that this TLR plays a unique role in host immune system in dairy calves (Malmuthuge et al., 2012).

Calf Performance. The impact of early milk quality on average daily gain (**ADG**), weight at day 30, mortality rates, and sexual maturity has been previously reported (Massimini et al., 2007; Mastellone et al., 2011; Hawk et al., 1953). Yang et al. (2015) also reported increased total protein serum levels in calves given higher quality colostrum (6.33 g/dL) when compared to lower quality colostrum calves (4.85 g/dL). Calves that ingested greater quality colostrum also

had an increased body weight at a mean rate of 2.2 kg by their 8th day of life when compared to calves administered lower quality colostrum who gained an average of 1.7 kg. In goats and buffalo calves it was determined that success of passive immunity was significantly correlated with average daily gain (r_p = 0.48; r_p = 0.72, respectively) and weight at day 30 (r_p = 0.56; r_p = 0.31). An increase in 1 mg/mL of IgG in serum was associated with an ADG of 0.005 kg/d and an increase in weight at day 30 of 0.185 kg (Massimini et al., 2007; Mastellone et al., 2011). This meant that increased milk quality had increased rates of passive immunity transfer and increased desirable calf performance traits. Also, increased rates of passive immunity transfer were associated with lower mortality rates as observed by Robinson et al. (1988). They found that passive immunity was most crucial between 70 d and 105 d after birth. Calves with < 18 mg/mL of total serum protein within the first 2 d after birth were twice as likely to die when compared to calves with > 18 mg/ml of total serum protein levels.

Taking into consideration the impact of passive immunity on calfhood scours, Hawk et al. (1953) and Menge et al. (1960) investigated possible associations with pubertal age and subsequent lactation performance. Weight gain was significantly different at 3 m ($P < 0.01$) and 6 m ($P < 0.02$) between animals that had developed calfhood scours and those that had not. Weight at 3 and 6 m was also found to be highly associated to pubertal age (r = -0.93; r = -0.54, respectively) by significantly delaying onset of puberty, in turn, inhibiting sexual development. Menge et al. (1960) observed a moderate negative correlation between age of puberty and 90-day milk production (r_p = -0.18; $P < 0.05$) and determined that there was an increase in milk production by 2.57 lb for every day earlier of pubertal age. Overall, quality of colostrum such as IgG content impacts passive immunity and overall health. This, in turn, can impact calf performance such as weight gain which can ultimately have detrimental effects such as delay in

pubertal age thus impacting sexual maturity. Subsequently, delays in pubertal age can have impacts on production traits such as those observed for 90-day milk production. This shows that factors such as early milk quality can have a cascade effect on health with subsequent, long-lasting effects on performance.

CONCLUSION

The relationships that exist between factors affecting early milk quality and subsequent effects on calves is an important topic that still needs to be explored more in depth. The livestock industry faces a big challenge with high heifer calf mortality rates. This is becoming costly and if not addressed can become inimical to the progress and growth of the industry as a whole. It has been recognized that early milk quality plays a very important role in the development of calves, and with new advances in technology and more research being conducted it is becoming increasingly possible to understand the complex relationships between quality, composition, and neonatal health.

At different stages of early milk production composition varies and this plays a role in determining the quality which can impact calf performance, calf gut development, and calf immune performance in a negative manner. There are also other contributing factors such as dam age, parity, breed, nutrition, season of calving, genetics, milk volume, and mode of birth that have proven to have an effect on quality of milk. However, factors such as genetics and mode of birth have been studied to a less extent in livestock animals and need further research. Studying these factors to a greater extent can potentially improve immune function such as making for a more efficient complement and antioxidant system, improve morphological and histological traits in calf gut development, and allow for improved performance traits such as better average daily gain, weight at 30 days, and even some reproductive traits as well as decrease mortality rates for pre-weaned calves.

REFERENCES

Abe, F., Ishibashi, N., & Shimamura, S. (1995). Effect of adminsitration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. *J. Dairy Sci., 78*(12), 2838-2846. doi:10.3168/jds.S0022-0302(95)76914-4

Agriland. (2019). Are your calves getting the right nutrtion from their transition milk? *Agriland*.

Akers, R. M. (Ed.) (2002). *Chapter 4: Milk Component Biosynthesis. Lactation and the Mammary* (First ed.).

Aragona, K. M., Chapman, C. E., Pereira, A. B. D., Isenberg, B. J., Standish, R. B., Maugeri, C. J., . . . Erickson, P. S. (2015). Prepartum supplmentation of nicotinin acid: effects on health of the dam, colostrum quality, and acquisition of immunity in the calf. *J. Dairy Sci, 99*, 3529-3538. doi:10.3168/jds.2015-10598

Ballard, O., & Ardythe, L. M. (2013). Human milk composition: Nutrients and bioactive factors. *Pediatr Clin North Am., 60*(1), 49-47. doi:10.1016/j.pcl.2012.10.002

Barrington, G. M., McFadden, T. B., Huyler, M. T., & Besser, T. E. (2001). Regulation of colostrogenesis in cattle. *Livestock Production Science, 70*, 95-104. doi:10.1016/S0301-6226(01)00201-9

Baumrucker, C. R., & Bruckmaier, R. M. (2014). Colostrogenesis: IgG1 Trancytosis Mechanism *J. Mammary Gland Biol Neoplasia, 19*, 103-117. doi:10.1007/s10911-013-9313-5

Besser, T. E., & Gay, C. C. (1994). The importance of colostrum to the health of the neonatal calf. *Vet Clin North Am Food Anim Pract., 10*(1), 107-117. doi:10.1016/s0749-0720(15)30591-0

Bolton, C., & Wright, G. P. (1936). THE ABSORPTION OF AMINO ACIDS AND THEIR DISTRIBUTION IN THE BODY FLUIDS. *Journal of Physiology, 89*(3). doi:10.1113/jphysiol.1937.sp003477

Cabrera-Rubio, R., Collado, M. C., Laitinen, K., Salminen, S., Isolauri, E., & Mira, A. (2012). The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Nutr, 96*, 544-551. doi:10.3945/ajcn.112.037382

Cho, Y., & Yoon, K. (2013). An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. *J. Vet. Sci, 15*(1), 1-17. doi:10.4142/jvs.2014.15.1.1

Dimbylow, L. (2019). Milk-Why its important in your babys diet.

Dizdar, E. A., Sari, F. N., Degirmencioglu, H., Canpolat, F. E., Oguz, S. S., Uras, N., & Dilmen, U. (2014). Effect of mode of delivery on macronutrient content of breast milk. *Journal of Maternal-Fetal & Neonatal Medicine, 27*(11), 1099-1102. doi:10.3109/14767058.2013.850486

Fernandez, L., Langa, S., Martin, V., Maldonado, A., Jimenez, E., Martin, R., & Rodriguez, J. M. (2012). The human milk microbiota: Origin and potential roles in health and disease. *Pharmacological Research, 69*, 1-10. doi:10.1016/j.phrs.2012.09.001

Gauthier, S. F., Pouliot, Y., & Maubois, J. L. (2006). Growth factors from bovine milk and colostrum: composition, extraction and biological activities. *Dairy Science & Technology, 86*(2), 99-125. doi:10.1051/lait:2005048

Gilbert, R. P., Gaskins, C. T., Hillers, J. K., Brinks, J. S., & Denham, A. H. (1988). Inbreeding and Immunoglobulin G1 Concentrations in Cattle. *J. Anim Sci., 66*(10), 2490-2497. doi:10.2527/jas1988.66102490x

Godden, S. M., Lombard, J. E., & Woolums, A. R. (2019). Colostrum Management for Dairy Calves. *Veterinary Clinics of North America: Food Animal Practice, 35*(3), 535-556. doi:10.1016/j.cvfa.2019.07.005

Guy, M. A., McFadden, T. B., Cockrell, D. C., & Besser, T. E. (1994). Regulation of colostrum formation in beef and dairy cows. *J. Dairy Sci, 77*(10), 3002-3007. doi:10.3168/jds.S0022-0302(94)77241-6.

Hawk, H. W., Tyler, W. J., & Casida, L. E. (1954). Some Factors Affecting Age at Puberty in Holstein-Friesian Heifers. *J. Dairy Sci., 37*(3), 252-258. doi:10.3168/jds.S0022-0302(54)91248-5

Heinrichs, J., & Jones, C. M. (2016, 05/05/2016). Milk Components: Understanding milk fat and protein variation in your dairy herd.

Hough, R. L., McCarthy, F. D., Kent, H. D., Eversole, D. E., & Wahlberg, M. L. (1990). Influence of nutritional restrictions during late gestation on production measures and passive immunity in beef cattle. *J. Anim. Sci., 68*, 2622-2627. doi:10.2527/1990.6892622x

Hurley, W. L. (1989). Mammary gland function during involution and the declining phase of lactation. *J. Dairy Sci, 72*, 1637-1646. doi:10.3168/jds.S0022-0302(89)79276-6

Jorgensen, M. W., Adams, A., Passille, A. M. d., Rushen, J., Salfer, J. A., & Endres, M. I. (2017). Mortality and health treatment rates of dairy calves in automated milk feeding systems in the Upper Midwest of the United States. *J. Dairy Sci., 100*, 9186-9193. doi:10.3168/jds.201713198

Kellems, R.O. and D.C. Church. 2002. Livestock Feeds and Feeding, 5 th ed. Prentice Hall, New Jersey .

Kleiber, M., Black, A. L., Brown, M. A., & Tolbert, B. M. (1952). Propionate as a precursor of milk constituents in the intact dairy cow. *J. Biol. Chem, 203*, 339-346.

Korhonen, H., Marnila, P., & Gill, H. S. (2000). Milk immunoglobulins and complement factors. *British Journal of Nutrition, 84*(1), S75-S80.

Loker, S. F., Bastin, C., Miglior, F., Sewalem, A., Fatehi, J., & Jamrozik, L. R. (2009). Genetic parameters of body condition score and milk production traits in Canadian Holsteins: . *Dairy Cattle Breeding and Genetics Meeting, Centre for Genetic Improvment of Livestock, University of Guelph (Canada)*, 11.

Malmuthuge, N., Meiju L., Patrick, F., Philip, J.G., and Guan L.L. (2012). Regional and age dependent changes in gene expression of Toll-like receptors and key antimicrobial defense molecules throughout the gastroinstetinal tract of dairy calves. *Veterinary Immunology and Immunopathology*. 146, 18-26. doi:10.1016/jvetimm.2012.01.010

Malmuthuge, N., Griebel, P. J., & Guan, L. L. (2015). The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. *Frontiers in Veterinary Science, 2*(35). doi:10.3389/fvets.2015.00036

Malmuthuge, N., & Guan, L.L. (2017). Understanding the gut microbiome of dairy calves: opportunities to improve early-life. J. Dairy. Sci. 100(7), 5996-6005. doi: 10.3168/jds.2016-12239

Mann, S., Yepes, L., Overton, T. R., Lock, A. L., Lamb, S. V., Wakshlag, J. J., & Nydam, D. V. (2016). Effect of dry period dietary energy level in dairy cattle on volume, concentration of immunoglobulin, insulin, and fatty acid composition of colostrum. *J. Dairy Sci, 99*, 1515-1526. doi:10.3168/jds.2015-9926.

Mastellone, V., Massimini, G., Pero, M. E., Cortese, L., Piantedosi, D., Lombardi, P., . . . Avallone, L. (2011). Effects of passive immunity trasnfer status on growth performance

in buffalo calves. *Asian-Australian Journal of Animal Sciences, 24*(7), 952-956. doi:10.5713/ajas.2011.10348

McGrath, B. A., Fox, P. F., McSweeney, P. L., & Kelly, A. L. (2016). Composition and properties of bovine colostrum: a review. *Dairy Sci & Technol, 96*, 133-158. doi:10.1007/s13594-0258-x

Menge, A. C., Mares, S. E., Tyler, W. J., & Casida, L. E. (1960). Some Factors Affecting Age at Puberty and the First 90 Days of Lactation in Holstein Heifers. *J. Dairy Sci., 43*(8), 1099-1107. doi:10.3168/jds.S0022-0302(60)90288-5

Merial. (2018). Assessing colostrum quality for optimal immunity in newborn calves. Retrieved from https://www.farmantibiotics.org/wp-content/uploads/2018/01/Assessing-colostrum-qualityplain-.pdf

Metka, M., & Nada, P. (1992). The role of exocytosis in the apocrine secretion of milk lipid globules in mouse mammary gland during lactogenesis. *Biol Cell, 75*(3), 211-216. doi:10.1016/0248-4900(92)90142-n

Missanjo, E., Imbayarwo-Chikosi, V., & Halimani, T. (2013). Estimation of genetic and phenotypic parameters for production traits and somatic cell count for jersey dairy cattle in zimbabwe. *ISRN veterinary science, 2013*, 470585. doi:10.1155/2013/470585

Moran, J. (2005). Tropical daiy farming: feeding managment for small holder fairy farms in the humid tropics. *Landlinks Press*, 312.

Morin, D. E., Constable, P. D., Maunsell, F. P., & McCoy, G. C. (2001). Factors associated with colostral specific gravity in dairy cows. *J. Dairy Sci, 84*, 937-943. doi:10.3168/jds.S0022-0302(01)74551-1

Morril, D. E., Conrad, P. D., Largo, A., Campbell, J., Quigley, J., & Tyler, H. (2012). Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *J. Dairy Sci, 95*, 3997-4005. doi:10.3168/jds.2011-5174

Muller, L. D., & Ellinger, D. K. (1981). Colostral immunoglobulin concentration among breeds of dairy cattle. *J. Dairy Sci., 64*(8), 1727-1730. doi:10.3168/jds.S0022-0302(81)82754-3

Nardone, A., Lacetera, N., Bernabucci, U., & Ronchi, B. (1997). Composition of Colostrum from Dairy Heifers Exposed to High Air Temperatures During Late Pregnancy and the Early Postpartum Period. *J. Dairy Sci., 80*(5), 838-844. doi:10.3168/jds.S0022-0302(97)76005-3.

National Research Council (US) Committee on Technological Options to Improve the Nutritional Attributes of Animal Products. Designing Foods: Animal Product Options in the Marketplace. Washington (DC): National Academies Press (US); 1988. Factors Affecting the Composition of Milk from Dairy Cows. Available from: https://www.ncbi.nlm.nih.gov/books/NBK218193/?report=classic

Nissen, E., Moberg, K. U., Svensson, K., Stock, S., Widstrom, A. M., & Winberg, J. (1996). Different patterns of oxytocin, prolactin but not cortisol release during breastfeeding in women delivered by Caesarean section or by the vaginal route. *Early Human Development, 45*(1), 103-118. doi:10.1016/0378-3782(96)01725-2

Nocek, J. E., Braund, D. G., & Warner, R. G. (1984). Influence of Neonatal Colostrum Administration, Immunoglobulin, and Continued Feeding of Colostrum on Calf Gain, Health, and Serum Protein. *J. Dairy Sci., 67*(2), 319-333. doi:10.3168/jds.S0022-0302(84)81305-3

Norman, L. M., Hohenboken, W. D., & Kelley, K. W. (1981). Genetic Differences in Concentration of Immunoglobulins G1 and M in Serum and Colostrum of Cows and in Serum of Neonatal Calves. *J. Anim. Sci., 53*(6). doi:10.2527/jas1982.5361465x.

Novo, S. M. F., Costa, J. F., Baccili, C. C., Sobreira, N. M., Maia, M. A., Leite, S. B. P., . . . Gomes, V. (2017). Specific immune response in neonate Holstein heifer calves fed fresh or frozen colostrum. *Pesq. Vet. Bras., 37*(12), 1385-1394. doi:10.1590/S0100-736X2017001200005

Nowak, W., Mikula, R., Zachwieja, A., Paczynska, K., Pecka, E., Drzaga, K., & Slosarz, P. (2012). The impact of cow nutrition in the dry period on colostrum quality and immune status of calves. *Polish Journal of Veterinary Sciences, 15*(1), 77-82. doi:10.2478/v10181-011-0117-5

Oikonomou, G., Teixeira, A. G. V., Foditsch, C., Bicalho, M. L., & Machado, V. S. (2013). Fecal Microbial Diversity in Pre-Weaned Dairy Calves as Described by Pyrosequencing of Metagenomic 16S rDNA. Associations of Faecalibacterium Species with Health and Growth. *PLOS ONE, 8*(4), e63157. doi:10.1371/journal.pone.0063157.

Osorio, J. S., Lohakare, J., & Bionaz, M. (2016). Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation. *Physiological Genomics, 48*(4), 231-256. doi:10.1152/physiolgenomics.00016.2015

Pakkanen, R., & Aalto, J. (1997). Growth Factors and Antimicrobial Factors of Bovine Colostrum. *Int. Dairy Journal, 7*, 285-297.

Perez M.D., Sanchez L., Aranda P., Ena J.M., Oria R., & Calvo M. (1990). Synthesis and evolution of concentration of beta-lactoglobulin and alpha-lactalbumin from cow and

sheep colostrum and milk throughout early lactation. *Cellular and Molecular Biology*. 36(2):205-212.

Petrini, J., Lung, L. H. S., Rodriguez, M. A. P., Salvian, M., Pertille, F., Rovadoscki, G. A., . . . Mourao, G. B. (2016). Genetic parameters for milk fatty acids, milk yield, and quality traits of Holstein cattle population reared under tropical conditions. *J. Anim Breed. Genet.*, 1-12. doi:10.1111/jbg.12205

Pillay, J., & Davis, T. J. (2019). Physiology, Lactation. *StatPearls.* Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK499981/

Pritchett, L. C., Gay, C. C., Besser, T. E., & Hancock, D. D. (1991). Management and production factors influencing immunoglobulin G concentration in colostrum from Holsteins cows. *J. Dairy Sci., 74,* 2336-2341. doi:10.3168/jds.S0022-0302(91)78406-3.

Quesnel, H., & Farmer, C. (2018). Review: nutritional and endocrine control of colostrogenesis. *Animal, 13,* S26-S34. doi:10.1017/S1751731118003555

Rainard P. (2017). Mammary microbiota of dairy ruminants: fact or fiction?. *Veterinary research*, *48*(1), 25. doi:10.1186/s13567-017-0429-2

Rocha, T. G., Franciosi, C., Nociti, R. P., Silva, P. C., Sampaio, A. A. M., & Fagliari, J. J. (2014). Influence of parity on concentrations of enzymes, proteins, and minerals in the milk of cows. *Arq. Bras. Med. Vet. Zootec, 66*(1), 315-320.

Roveglia, C., Costa, A., Penasa, M., Finocchiaro, R., Marusi, M., Lopez-Villalobos, N., & Cassandro, M. (2017). *Genetics of milk traits including lactose and somatic cell score in Italian Jersey cows.* Paper presented at the Proceedings of the World Congrees on Genetics Applied to Livestock Production.

Ruiz L., Delgado S., Ruas-Madiedo P., Sanchez B., & Margolles A. (2017). Bifidobacteria and their molecular communication with the immune system. Front Microbiol. 8: 2345. doi:10.3389/fmicb.2017.02345

Sano, H., Ambo, K., & Tsuda, T. (1985). Blood glucose kinetics in whole body and mammary gland of lactating goats exposed to heat. *J. Dairy Sci., 68*(10), 2557-2564. doi:10.3168/jds.S0022-0302(85)81137-1

Silva-del-Rio, N., Rolle, D., Garcia-Munoz, A., Rodriguez-Jimenez, S., Valldecabres, A., Lago, A., & Pandey, P. (2017). Colostrum immunoglobulin G concentration of multiparous Jersey cowsat first and second milking is associated with parity, colostrum yield, and time of first milking, and can be estimated with Brix refractometry. *J. Dairy Sci., 100*, 5774-5781. doi:10.3168/jds.2016-12394

Shennan, D. B., Millar, I. D., & Calvert, D. T. (1997). Mammary-tissue amino acid transport. *Nutrition Society, 56*, 177-191.

Soufleri, A., Banos, G., Panousis, N., FleKaantouris, D., Arsenos, G., & Valergakis, G. E. (2019). Genetic parameters of colostrum traits in Holstein dairy cows. *J. Dairy Sci., 102*, 11225-11232. doi:10.3168/jds.2019-17054.

Sovereign Laboratories. (2017). Benefits of Lactoferrin in Bovine Colostrum. Retrieved from https://www.sovereignlaboratories.com/blog/benefits-lactoferrin-bovine-colostrum/

Stein, T., Salomonis, N., & Gusterson, B. A. (2007). Mammary Gland Involution as a Multi-step Process. *J. Mammary Gland Biol Neoplasia, 12*, 25-35. doi:10.1007/s10911-007-9035-7

Stelwagen, K., Carpenter, E., Haigh, B., Hodgkinson, A., & Wheeler, T. T. (2008). Immune components of bovine colostrum and milk. *J. Anim Sci., 87*(1), 3-9. doi:10.2527/jas.2008-1377

Toscano, M., De Grandi, R., Peroni, D. G., Grossi, E., Facchin, V., Comberiati, P., & Drago, L. (2017). Impact of delivery mode on the colostrum microbiota composition. *BMC microbiology*, *17*(1), 205. https://doi.org/10.1186/s12866-017-1109-0

Tucker, H. A. (1980). Physiological control of Mammary Growth, Lactogenesis, and Lactation. *J. Dairy Sci., 64,* 1403-1421.

USDA. (2011). Cattle and calves nonpredator deathloss in the United States. *USDA-APHIS*.

Waters, S. B., & Rillema, J. A. (1988). Effect of prolactin on enzymes of lipid biosynthesis in mammary gland explants. *The American Physiological Society*.

Watson, C. J. (2006). Involution: apoptosis and tissue remodelling that convert the mammary gland from milk factory to a quiescent organ. *Breast Cancer Research, 8,* 203. doi:10.1186/bcr1401

Yamanaka, H., Hagiwara, K., Kirsawa, R., & Iwai, H. (2003). Proinflammatory Cytokines in Bovine Colostrum Potentiate the Mitogenic Response of Peripheral Blood Mononuclear Cells from Newborn Calves through IL-2 and CD25 Expression. *Microbiology and Immunology, 47,* 461-468. doi:10.1111/j.1348-0421.2003.tb03371.x

Yang, M., Zhou, Y., Wu, Z. H., Li, S. L., & Cao, Z. J. (2015). Colostrum quality affects immune system establishment and intestinal development of neonatal calves. *J. Dairy Sci., 98*(10), 7153-7163. doi:10.3168/jds.2014-9238.

Yang, Z., Jiang, R., Chen, Q., Wang, J., Duan, Y., Pang, X., Jiang, S., Bi, Y., Zhang, H., Lönnerdal, B., Lai, J., & Yin, S. (2018). Concentration of Lactoferrin in Human Milk and Its Variation during Lactation in Different Chinese Populations. *Nutrients*, *10*(9), 1235. https://doi.org/10.3390/nu10091235

CHAPTER 2: MODE OF DELIVERY AND TRANSITION MILK COMPOSITION IMPACT RUMEN MICROBIOME COMPOSITION IN PRE-WENAED BEEF CALVES

Abstract

Early milk components provide a continual inoculation of microbial species within the calf gut. The gut microbiome stimulates development of epithelial tissue and immune cell differentiation. Certain components within early milk act as immunomodulatory agents that can be beneficial to the calf's early development. Therefore, it is important to determine which factors can significantly impact early milk composition. It is not known how the maternal environment influences the composition of early milk, nor is it known how these changes in composition impact the abundance of microbial species abundance in the calf gut. We hypothesized that mode of birth would impact early milk composition, and, in turn, influence the microbial taxa in the calf rumen. The objectives of this research were to: 1) determine the relationship between mode of birth and milk composition and 2) identify how changes in milk composition impact the abundance of microbial phyla in the calf rumen. Transition and mature milk were collected from Charolais (n =23) and Angus (n = 15) dams on d 1, 3, and 28 post-partum that underwent vaginal (**VD**) or cesarean-section (**CD**) delivery. Milk samples were analyzed for true protein, lactose, somatic cell count, and fatty acid concentrations. Extracted DNA from d 1, 3, and 28 calf rumen fluid were used for metagenomic sequencing. Samples were run on the Illumina HiSeq 2500 platform as paired end reads. Individual milk components were regressed on mode of birth for each day using the GLM procedure in SAS. Because sequence data is non-normally distributed, a non-parametric regression analysis was conducted using the GENMOD procedure in SAS; whereby, milk composition parameters were regressed against transformed phyla level microbial counts. Vaginal delivery dams were more likely to have increased ($P \leq 0.001$) protein, solids non-fat, and lactose on d 1 and 3, but decreased ($P < 0.001$) urea concentrations. Similarly, short, medium, and long-chain fatty acids were increased ($P \leq 0.05$) in VD d 3 milk. True protein

elicited a decrease ($P \leq 0.05$) in rumen fluid abundances of Actinobacteria and Proteobacteria on d 1; whereas, both solids non-fat and lactose were associated with an increased ($P \leq 0.05$) response in d 1 transition milk. There were no significant results for d 28 of sampling for milk components, fatty acids, or microbial phyla. Based on these results, we suggest that mode of birth influences protein concentrations in early milk. Though Actinobacteria abundances appear to be responsive to milk composition changes when compared to other phyla, the overall dynamics of the calf rumen microbiome are relatively stable in response to changes in milk composition at the phyla level.

Keywords: beef cattle, milk composition, rumen microbiome

INTRODUCTION

Transition and mature milk are terms referring to characteristics of milk produced post-partum. Transition milk is synthesized and secreted after colostrum but before mature milk. In cattle, transition milk is typically produced for up to 72h after calving and is dependent on milk synthesis and removal rate. Increased frequencies of milk synthesis, milk removal, or both, hasten progression to mature milk. Increasing evidence in humans and livestock suggest that abundance of milk components (triglycerides, lactose, specific mammary proteins (e.g., caseins, alpha-; lactalbumin, and beta-lactoglobulin), non-specific mammary proteins (e.g., immunoglobulins, transferrin, lactoferrin), hormones, volatile compounds, (beta-hydroxybutyrate (**BHB**), Acetone), and Somatic Cell Score (**SCS**) in transition and mature milk can be affected by biological and environmental elements. This would include elements such as species, breed, maternal factors (i.e. dam age, parity, genetics), and pathogenic bacteria (Langer, 2009; Fahmy, 1972; Cunningham et al., 2018; Bagnicka et al, 2011). Changes in early milk composition attributed to the elements mentioned above could also have subsequent effects on the development on early gut microbiome (Elsen et al., 2019).

The gut microbiome in the calf starts to develop in utero, followed by initial colonization through the birthing process, and then continues developing through exposure to its environment (Dunn et al., 2017). The gut is initially colonized by bacteria that utilize available oxygen by NADH oxidase enzymes which reduces molecular oxygen to hydrogen (Ahn et al., 2007). This creates an anaerobic environment that is needed for some microbial species such as Bifidobacterium and Bacteroidetes to survive. Multiple factors (successful microbial adhesion, mode of delivery, and vaccination program of the dam) can impact these normal processes and, in turn, the development of a healthy microbiome for the calf. We know that the establishment of a healthy pre-ruminant microbiome plays an important role in survivability and susceptibility to

enteric infections, it also has the potential to set the stage for later-life feed efficiency (Powell et al., 2018), plays a critical role in the development of mucosal immune system, and immunological tolerance (Malmuthuge, et al., 2015). As mentioned previously there are multiple factors that can affect the normal development of rumen microbiome, however, little is known how composition of the milk ingested by calves impacts microbial abundance of the rumen. Initially, the pre-weaned calf is born with an undeveloped rumen. Calves utilize the esophageal groove for milk to bypass digestion in the rumen and reach the abomasum. However, throughout this process there is some leakage of the milk into the rumen that is speculated to have early impacts on the rumen microbiome. Therefore, it is important to study changes in milk composition and its subsequent effects on the early rumen microbiome development in calves. Factors affecting milk composition are attributed to the dam and are known as maternal factors. Maternal factors impacting biological processes include age, breed, parity (i.e. primiparous and multiparous), and mode of delivery. In humans, research has focused on the effects of mode of delivery on maternal milk composition. Dizdar et al. (2013) reported increased protein levels in mothers that delivered vaginally when compared to those that had cesarean deliveries. There is limited research in cattle on impacts of mode of birth on early milk composition. Thus, our objectives were to 1) determine the relationship between mode of birth and milk composition and 2) identify milk components or biological factors that impact microbial phyla abundance in the calf gut. With these objectives in mind we aimed to further elucidate the role of milk components in the survival and propagation of microbial phyla within the calf gut and the subsequent contributions to the overall health and development of the calf. We hypothesized that mode of birth (i.e. vaginal delivery versus cesarean section) impacts early milk composition, and, in turn, would influence the microbial phyla in the calf gut.

Materials and Methods

Animal Procedures:

Cow management and sample collection. All animal procedures were approved by the University of Wyoming Animal Care and Use Committee (20150903KC00194-01). Multiparous Angus and Charolais dams were used in this study. Cows were randomly divided into experimental groups. Cows were bred by natural service and at 250 d of gestation cows were monitored closely for signs of parturition as previously described by Cunningham et al. (2018). Experimental groups were 1) vaginal delivery (ANG n = 12; CHAR n = 13) our control, and 2) cesarean section (ANG n = 3; CHAR n = 10). The VD cows calved naturally with no intervention. Cesarean delivery cows were closely monitored, and cesarean section was performed by a licensed veterinarian when the cow presented signs of parturition. An epidural was administered utilizing 6-10 mL of lidocaine and injecting in tail head and cesarean performed using methods by Cunningham et al. (2018). Briefly, cesarean section was performed at the left paralumbar fossa after the site was clipped of hair and surgically scrubbed with betadine. After 10-20 mL of lidocaine was used to block the site using an inverted L line. An incision was made vertically in the middle of the paralumbar fossa, starting approximately 10 cm central to transverse process of the lumbar vertebrae and continuing ventrally, far enough to allow calf removal. A Bovikalc bolus (Boehringer Ingelheim, Duluth, GA) was utilized prior, immediately after, and 48 hrs post cesarean section in order to avoid calcium deficiency. After parturition, both milk samples and rumen fluid samples were collected from cows and calves respectively at 24 hrs (d 1) and d 3 and 28. Milk samples were analyzed for components such as fat, true protein, solids non-fat (SNF), lactose, somatic cell count, acetone, β-hydroxybutyrate (BHB), and urea by the Dairy Herd Improvement Association (DHIA; Radford, VA). Fatty acids were also analyzed using MilkoScan^{TM} FT+/FT 6000 fatty acid prediction models.

Calf management and sample collection. After parturition, all experimental groups were spatially separated. Dams were allowed to rear their calves until weaning (205 d). Rumen fluid was collected from each calf via oral lavage at d 1, 3, and 28 d post-partum. Briefly, using methods described by Lodge-Ivet et al. (2009) a vinyl tube (0.9 m in length, 1.25 cm outer diameter) was lubricated and passed into the rumen, a syringe attached to one end was used to suction approximately 20-30 mL of rumen fluid. Rumen fluid was immediately frozen on dry ice and stored at -80° C. Microbial DNA was extracted from rumen fluid obtained at d 1,3, and 28 from VD (n=17) and CD calves (n=7) using the methods previously described by Yu and Morrison (2004) and Cunningham et al. (2018). Briefly, lysis buffer (500mM NaCl, 400mM Tris-HCl, 50mM EDTA, 4% SDS) was added to 0.25 g of rumen fluid and sterile zirconia (0.3 g of 0.1 mm) and silicon (0.1 g of 0.5 mm) beads. Samples were homogenized using a Mini-Beadbeater-8, incubated at 70° C for 15 min, and centrifuged for 5 min at 4° C. Homogenization, incubation, and centrifugation were repeated and the supernatant pooled. In order to obtain nucleic acid precipitation, 260 µL of ammonium acetate was added to each lysate, followed by a 5 min of incubation, centrifugation at 4° C for 10 min, and aliquoting of supernatant into two 2 mL flat cap tubes. Next, 600 L of isopropanol was added to each tube and mixed, then incubated for 30 minutes and centrifuged again at 4° C for 15 min. Supernatant was removed and pellet washed with 70% ethanol followed by pulse centrifugation. After all ethanol was removed and pellets dried, pellets were then resuspended in 100 ul of TE buffer. DNA was further purified using QIAamp Stool Mini Kit (Qiagen, Santa Clarita, CA, USA). Kit protocol was followed with the exception of the addition of 200 µL buffer EB instead of buffer AE for elution for protein removal and further purification of DNA. The concentration of DNA was then measured using a Nanodrop 1000 spectrophotometer (ThermoScientific, Wilmington, DE, USA). Quality of the

DNA was determined using gel electrophoresis and samples reached a 260/280 > 1.5. Good quality samples were diluted to 5μg total in 100 ul aliquots and sent for metagenomic sequencing at the University of Missouri DNA Core Facility in Columbia, MO USA.

Library Preparation and Metagenomic Analysis. Libraries were constructed using the manufacturer's (Illumina, San Diego, CA, USA) protocol along with Illumina's TrueSeq DNA PCR-Free sample preparation using 1 μg of initial genomic DNA in order to produce a 350bp average insert. Purified libraries were then quantified with a Qubit assay, and a Fragment Analyzer (Advanced Analytical Technologies, Inc, Ankeny, IA, USA) was used to determine fragment size. Libraries were diluted, followed by samples being multiplexed and run through Illumina HiSeq 2500 platform for sequencing. Metagenomic sequences were quality filtered using Metaxa2. Briefly, hidden Markov models using HMMER identified the conserved regions of the small subunit by aligning Metaxa2 curated database. Metaxa2 allowed us to identify taxonomic assignment and then simply down to abundances at the phyla level (Cunningham et al., 2018).

***Statistical analyses*:**

Descriptive Statistics. A PROC Mean in SAS 9.4 was used to determine the mean, standard error, min, and max for milk components and fatty acids in early milk. This was done on the full dataset (n= 38) without distinguishing between treatment group, day of sample, or breed**.**

Milk composition. Fatty acids were converted to g/ g total fatty acids (**TFA**) using a milk prediction model that incorporated milk fat percentage and conversion factor of 0.95 (from total fat to total fatty acids) into the equation: $\frac{\text{g Fatty acid /g 100 milk}}{(\text{total Fat\%})(0.95)} \times 100$ (FOSS MilkoScan FT+/FT 6000 Application Note). The Box Cox procedure was then used to assess normalization for milk components (fat $\lambda = 0.25$, true protein $\lambda = -0.50$, SNF $\lambda = -1.50$, Lactose $\lambda = 3$, SCS $\lambda = 0$, urea λ

= 0.25) and fatty acids (C14 λ = -1.25, C16 λ = -1.25, C18 λ = 1.25, C18:1 λ = 1.25, LCFA λ = 1.25, MCFA λ = 0.50, MUFA λ = 1.25, PUFA λ = 0, TUFA = 1.25, SCFA λ = 2.75). Acceptable lambda values range from -3 to 3. A regression analysis was then performed in SAS 9.4 to determine the relationship of the main effect of mode of delivery (i.e. vaginal versus cesarean section) on transition and mature milk composition. Mode of delivery was measured as a categorical variable, and then incorporated into the model as a binary variable with (1=VD, 0=CD) in order to facilitate SAS in reading the data. The relationship of mode of delivery was tested against true protein, lactose, SNF, SCS, urea, fat, long-chain fatty acids, short-chain fatty acids, and medium-chain fatty acids. An alpha of ≤ 0.05 was used to establish significance. The effect of day was independently tested to determine the direct relationship between milk composition and rumen fluid microbial phyla. The interaction between day and mode of delivery was tested but not included as it was found to be statistically insignificant. Day as a predictor was also not incorporated into the model since there were limited number of CD requiring further sampling which was prohibitive.

Influence of milk composition on rumen microbiome. Microbial phyla read counts were converted into proportions, due to the non-linear nature of the data, and then a Box Cox procedure was used to determine appropriate transformation of phyla (Actinobacteria λ= 0.25, Bacteroidetes λ= 1.75, Firmicutes λ= 0, Proteobacteria λ= -0.25). Effect of changes in milk composition on the calf gut microbial taxa was determined by performing a multiple linear regression (**MLR**) using PROC Reg in SAS 9.4. This allowed us to determine the relative contribution of each predictor to the total variance in phyla abundance. A subset of the data was used for this analysis which incorporated the sequencing data from rumen fluid samples from d

1, 3, and 28. Only components found to be significantly impacted by mode of birth were included in the MLR model. The model used was:

1) $$Y_i = \beta_0 + \beta_{x1} + \beta_{x2} + \beta_{x3} + \beta_{x4} + \beta_{x6} + \beta_{x7} + \beta_{x8} + \beta_{x9} + \beta_{x10} + \beta_{x11} + \beta_{x12}$$

The 4 phyla were used as dependent variables (Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria) and are represented by Y_i, β_0 is the intercept, $\beta_{1...12}$ represent parameter estimates, and $x_{1..x12}$ the independent variables which are the milk components and fatty acids. Microbial phyla were incorporated into the model with fixed effects including breed, mode of birth, and sex. However, they were found to be non-significant and removed from the model. An alpha of ≤ 0.05 was used to establish significance. The relationship between mode of birth and the rumen microbiome has been previously reported by Cunningham et al. (2018).

Results

Descriptive Statistics. Based on the results, the mean concentration of fatty acids was within an acceptable range and can be found on **Table 2.1**. Reference values for acceptable fatty acid range can be found in the FOSS MilkoScan FT+/FT 6000 Application Note booklet (FOSS, 2017). For milk components some deviations from the normal range were observed. Mean true protein (5.91 %) and fat (4.45 %) being higher than the average for typical bovine milk. However, this is in part because we did not separate descriptive statistics by day, and samples from d 1 are expected to have higher values of fat and protein which can shift mean values to being higher than expected. Standard errors were also small which signifies a representative sample of the population.

Mode of delivery. Milk composition changes for VD and CD dams is reported in Table **2.2**. Dams that delivered vaginally had a decrease in urea by 0.44 mg/dl ($P < 0.05$) when compared to cows that delivered through cesarean section on d 1 and 3 milk samples but were not different on d 28 ($P > 0.05$). However, dams delivering vaginally had an increase ($P < 0.05$) in true protein

by 0.09%, SNF by 0.009%, and lactose by 26% when compared to CD for d 1 and 3 milk samples, with no differences in d 28 ($P > 0.05$).

Fatty acid profiles for transition and mature milk are reported in **Table 2.3**. There were no fatty acid profiles for d 1 sampling, therefore, analysis was only performed for d 3 and 28. Dams milk on d 3 of sampling had significant ($P < 0.05$) fatty acid profile differences (C14:0, C16:0, C18:0, C18:1, LCFA, MCFA, PUFA, TUFA, and SCFA) in cows that delivered vaginally when compared to those delivering through cesarean section. While there are multiple fatty acid types in our analysis, we can divide them up into three main categories: short-chain fatty acids (**SCFA**), medium-chain fatty acids (**MCFA**), and long-chain fatty acids (**LCFA**). For short chain fatty acids, which are chains containing up to 6 carbons, we observe a 54% increase ($P < 0.05$) in milk of dams that had VD when compared to CD. Medium-chain fatty acids, containing 7-12 carbons, showed a slight increase ($P < 0.05$) by 0.03% in VD versus CD. On the other hand, long-chain fatty acids, which include C14:0, C16:0, C18:0, and C18:1, showed a 31% increase ($P < 0.05$) in VD versus CD. Comparing these three groups, we concluded that mode of birth appears to impact the concentration of milk SCFA predominantly since it surpasses the concentration of milk MCFA by 53% and LCFA by 23%.

There were no significant differences found in the milk composition and fatty acid profile of cows that delivered vaginally compared to those that delivered through cesarean section on d 28 of sampling.

***Rumen microbiome*.** Results for multiple linear regression (**MLR**) for Actinobacteria regressed on milk components and fatty acids are in **Table 2.4**. Within transition milk, abundance of actinobacteria decreased by 2% in association with a one-unit change in our predictor true protein ($P < 0.05$).On the contrary, Actinobacteria abundance increased by 19% and 0.002% in

association with a one unit change of SNF and lactose ($P < 0.05$) on d 1 of sampling. On d 3 of sampling, Actinobacteria decreased per one-unit change in Urea ($P < 0.05$) but increased per one-unit change in C18:1 ($P < 0.05$). There were no significant changes in abundance of Actinobacteria due to changes in milk composition on d 28 of sampling.

Bacteroidetes regressed on milk components and fatty acids are reported in **Table 2.5**. For d 3 of sampling, Bacteroidetes abundance decreased by 0.01% per one-unit change in concentration of C18:1 ($P < 0.05$). No significant ($P > 0.05$) changes in abundance of Bacteroidetes was found for d 1 and 28 samples due to changes in milk composition.

Results for Firmicutes regressed on milk components and fatty acids are provided in **Table 2.6**. For d 1 samples, Firmicutes abundance decreased by 0.04% per one-unit change in concentration of lactose ($P < 0.05$). Firmicutes abundance increased in association with a one-unit change in concentration of C18:1 ($P < 0.05$) for d 3. There was no statistical significance in phyla abundance for d 28 samples.

Results for Proteobacteria regressed on milk components and fatty acids are reported in **Table 2.7**. For d 1 samples, Proteobacteria abundance decreased by 26% per one-unit change in concentration of true protein ($P < 0.05$). Also, Proteobacteria abundance increased ($P < 0.05$) in by 213% and 0.02% due to a change in one unit of SNF and lactose, respectively. There was no significant change in phyla abundance for d 3 and 28 samples. Results indicated statistically significant changes in phyla abundance for d 1and 3 samples. Even though significant changes to microbial abundance are present the overall dynamics of the calf rumen microbiome are relatively stable in response to changes in milk composition and fatty acid profile at the phyla level.

DISCUSSION

The first weeks after a calf is born marks a crucial time period in which the microbial population inoculates the gut. Establishment of a healthy gut microbiome is critical for the development of the mucosal immune system within the digestive system. Colonization of the gut is affected by multiple factors: host, microbial, and environmental (Malmuthuge et. al, 2015). Studies examining the role of external factors such as maternal microbiota, diet, and mode of birth show the ability of the gut microbiome to be altered by interfering with normal physiological processes (Bokulich et al., 2016). Early milk composition and fatty acid profile for d 1, 3, and 28 were within normal range for this study. Milk composition and fatty acid profile differ over time. These differences in composition are mostly observed in protein, lactose, SCFS, MCFA, and LCFA. From 24 to 72 h post-partum transition milk is being produced; transition milk contains increased amounts of proteins (i.e. whey and caseins) to provide immune support to neonates. Lactose is greatly responsible for 50 % of osmotic pressure in milk and allows for the influx of water into the mammary gland thus increasing volume (McGrath et al., 2016). This is why there are differences in protein and lactose content of milk overtime. During the transition milk stage protein levels are still high and increase the density of milk, therefore, less lactose is available. Furthermore, lactose content increases during mature milk production which is due to the decrease in protein levels and density. This allows for increased volume of milk to provide nutrients for the developing calf (McGrath et al., 2016). Additionally, SCFA, MCFA, and LCFA varied over time. These changes can be attributed to lactation stage differences. Samples taken early on during onset of lactation have less LCFA because de novo fatty acid synthesis is hindered due to negative energy balance of dams during the first weeks after parturition (Hanus et al., 2018). Conversely, SCFA and MCFA increase as dam start producing mature milk and is no longer in negative energy balance (**NEB**). Fatty acids such as MUFA and PUFA remained

stable throughout the study which is observed in literature as well (Hanus et al., 2018). Additionally, milk composition and fatty acid profile stabilized after the first week of the study. This goes back to the issue of NEB in lactating cows. Cows on average undergo NEB for 30 d post-partum, after this period the energy expended is for milk production and maintenance needs of the cow (Hanus et al., 2018). This explains why at d 28 no significant changes were observed in milk composition, fatty acid profile, or impacts to the rumen microbiome. Finally, reported values for milk composition and fatty acid profile from literature resembled those observed in this study thus the sample is representative of the Charolais and Angus population (FOSS, 2017; Jastrzebska et al., 2007).

Impact of mode of birth on milk composition has been extensively studied in humans it remains relatively unexplored in cattle. Dizar et al. (2014) demonstrated that macronutrients such as proteins in early milk were impacted by mode of delivery. Women that delivered vaginally had increased levels of protein in their milk when compared to cesarean deliveries while no carbohydrate or fat differences were observed. Additionally, fatty acid profile changes due to mode of birth have been observed (Sinanoglou et al., 2017). Myristic acid (C14), linoleic acid (C18:3), and PUFA increases in vaginal dams compared to cesarean delivery dams but shows a decrease in Oleic acid (C18:1) and MUFA in vaginal when compared to cesarean. Our data showed significant differences in composition of transition and mature milk depending on the mode of birth (VD vs CD).While these studies were conducted in humans, this does serve as a preliminary guideline for species that have not been vastly studied such as livestock. However, since CD are not common in livestock, differences in early milk composition attributed to mode of birth do not carry as much importance as impact of maternal factors. Dam age, parity, dry-

period length, diet, and season of calving are considered maternal factors and can all impact early milk composition to varying degrees (Puppel et al., 2019).

Milk composition. Milk protein, lactose, and fatty acids all have unique biosynthetic pathways that allow them to be transported into the mammary gland tissue and subsequently into milk (Akers, 2002). Protein synthesis is heavily dependent on the availability of energy content in the diet as well as amino acid (**AA**) content. Once protein is ingested it can be 1) degraded into volatile fatty acids (**VFA**) in the rumen as a byproduct of microbial fermentation and then absorbed by papillae or 2) ruminal microbes can utilize AA rich proteins for energy and incorporate them into microbial crude protein. The microbial proteins then travel to the abomasum followed by the small intestine where they empty from intestinal villi and enter venous circulation and later reach arterial circulation. At this point, proteins have been degraded into amino acids which can be transported into the mammary cell via active transport through transporters such as gamma glutamyl transpeptidase (Bionaz et al., 2012). Once the amino acids are in the cells, they can be used to make the primary milk proteins whey and casein. One explanation for the differences we observe in protein levels between VD and CD could be because of the relationship between protein production and TF. Also, maternal factors such as parity and season of calving could also contribute to differences in early milk protein concentrations. Dams in their second parity had significantly less ($P < 0.05$) protein content (13.0%) in their milk when compared to dams their third through fifth parities (14%-15%). Additionally, season of calving also impacted protein content, with dams calving in the spring having less protein (13.2%) in their milk when compared to dams that calved in the fall, winter, or summer (13.9%-14.1%; Dunn et al., 2017).

Lactose is the major osmole of milk; therefore, milk yield greatly depends on the synthesis of lactose. Lactose synthesis is dependent on glucose availability. Mammary tissue of lactating animals utilizes approximately 20% of blood glucose in order to synthesis lactose. Lactose in milk is synthesized by utilizing dietary carbohydrates (**CHO**; Osorio et al, 2016). The rumen microbes ferment the CHO into an assortment of end products with one being propionate. Propionate travels to the liver and undergoes gluconeogenesis which forms glucose. Glucose exits the liver and travels to the mammary cell where it is transported by *GLUT1,* a facilitated glucose transporter. Once inside the cell, glucose is sent to the Golgi where, in the presence of α-lactalbumin, it undergoes a conformational change and is converted to galactose. The process of converting glucose to galactose depends on β 1,4-galactosyltransferase, which is activated by α -lactalbumin. After galactose is formed, glucose and galactose combine to make lactose. The gene *B4galt1* encodes for β 1,4-galactyltransferase and in the event there is no α-lactalbumin, it could significantly impact gene activity (Osorio et al, 2016). In our study, protein concentration in CD cows decreased compared to VD cows. Based on these results and previous research, we suggest that the disruption in protein synthesis in CD cows had a cascade effect decreasing the amount of lactose in CD cows. However, no definitive conclusion can be drawn from this since further studies are needed to determine if this is the exact mechanism that accounts for the differences in lactose concentration for our experimental groups. Additionally, a decrease in lactose can also be attributed to maternal factors such as parity and calving season. While all the cows in this study were multiparous there is significant differences in lactose concentration by parity. Dams in their second parity had greater lactose concentration in early milk when compared to fourth and fifth parity damns (Dunn et al., 2017). Furthermore, lactose content in milk of dams that delivered in the spring were higher (2.8%) when compared to dams that calved during the fall, winter, and

summer (2.6%). These results are opposite to what is observed for protein content, which further supports the inverse relationship that exists between protein and lactose content (Dunn et al., 2017).

Fatty acids in milk are synthesized from two precursors 1) dietary fat and 2) dietary fiber. From each precursor different types of fatty acids are formed. Fermentable dietary fiber produces SCFA and MCFA while dietary fat gives rise to LCFA, PUFA, and other saturated fatty acids. When a cow ingests fermentable fibers, the rumen microbes ferments them to primarily acetate which is absorbed through the rumen papillae. Acetate is then in the bloodstream and must diffuse through mammary secretory cell. Once diffused, it can be used for fatty acid synthesis occurring in the cytoplasm of the cell. Acetate is then converted to Acetyl coA with the help of enzyme acetyl coA carboxylase. Afterwards, acetyl coA is acted upon by enzyme fatty acid synthase to make malonyl coA. Malonyl coA then goes through the malonyl coA pathway which allows for the addition of carbons on the growing fatty acid chain (Zhu and Luo, 2017).

Conversely, LCFA and PUFA come from dietary fat. Microbes in the rumen utilize the fiber and convert unsaturated fatty acids into saturated fatty acids by the addition of a H^+ in a process called biohydrogenation. These saturated fatty acids cannot be absorbed through rumen papillae; therefore, they flow to the small intestine for absorption. In the bloodstream they travel to the mammary gland as free fatty acids and are diffused into the tissue. Once inside they will be assembled into triglycerides (C16:0 and C18:1) near the rough endoplasmic reticulum. The process of fatty acid synthesis, similar to protein and lactose, can be impacted by hormone disruption due to differences of mode of birth. It is possible that the disruption in hormones could affect the integration of acetate into the fatty acid synthesis process via dietary fibers as well as alter the synthesis of lipids which affects production of fatty acids via dietary fats

(Waters and Rillema, 1988). This could explain why fatty acids were decreased in CD cows and why certain types of fatty acids such as SCFA were in greater quantities. Additionally, diet fed to the cow can also affect the fatty acid profile of early milk. Investigators have observed that disproportionate ratios of roughage and concentrates affect the production of acetate, thus, disrupting fatty acid synthesis. Also, increased biohydrogenation of PUFA in the rumen of the cow can also impact fatty acid synthesis by increasing levels of C18:1 (Collomb et al., 2008).

Rumen ecology response to milk composition. There are four major phyla of the gut microbiota: Actinobacteria (8.2%), Bacteroidetes (27.8%), Firmicutes (38.8%), and Proteobacteria (2.1%; Argenio & Salvatore, 2015) each playing a role in the development of a healthy gut; however, there is limited research on the impacts of early milk composition changes on the gut microbiota. The current study elucidates such relationships by showing changes in the abundance in these four major phyla over time. Actinobacteria plays a major role in the maintenance of gut homeostasis and bacterial disease control (Binda et al., 2018). Bacteroidetes can control their environment by interacting with the host immune system in order to control competing pathogens and are highly proficient at sensing available nutrients in their environment (Wexler, 2007). They can ferment polysaccharides and indigestible carbohydrates to produce SCFA (Birg et al., 2019). Firmicutes assist with energy reabsorption (Krajmalnik-Brown et al., 2012) and utilize carbohydrates as an energy source to produce butyrate (Sheridan et al., 2016). Proteobacteria play a role in the healthy gut maintenance and preventing metabolic disorders and inflammatory conditions (Rizzati et al., 2017). From this study, Actinobacteria and Proteobacteria were found to be more responsive to changes in early milk composition indicating that these phyla possibly utilize or rely on certain milk components as substrates to assist them in performing other functions such as the ones mentioned previously.

Another thing to consider is stability of the calf rumen microbiome in the first few weeks after birth and how this relates to the results from our study. Alipour et al. (2018), observed the first week of life marked by fluctuations in the gut microbiota abundance of phyla such as Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes. Actinobacteria abundance decreased from birth to d 7 and Proteobacteria was not present by d 7. Firmicutes and Bacteroidetes increased by d 7 with d 7 perinatal calf microbiota appearing to stabilize. Another study by Malmuthuge et al. (2019) investigated the composition of calf rumen microbiome within the first 6 wks of life. They found the same 4 major phyla (Bacteroidetes, Actinobacteria, Proteobacteria, and Firmicutes) present in their samples. From birth through wk 6 they observed an increase in Firmicutes and Bacteroidetes with abundance stabilizing around 3 wks. They also observed a decrease in Actinobacteria abundance from birth to wk 1, but it then increased by wk 3 and remained stable. Proteobacteria was present in very small amounts during the first week of life and then increased by wk 3 and remained stable. Results for both studies are similar and show a rumen microbiota very similar to the fecal microbiota of an adult cow within 3 weeks post-partum. This shows the initial signs of the calf gut microbiota transitioning to be able and support digestion of more nutrient dense, solid feeds reared towards feeding the microbes of what will soon be a fully functional rumen allowing for the production of VFA and degradation of nutrients needed for other biosynthetic processes. We can observe from our results that by d 28 there were no significant changes to the phyla abundance with most of the changes being observed on d 1 or 3.

CONCLUSION

Despite intensive investigation in humans on the impact of mode of birth on milk composition and volume; the relationship between effects of mode of birth on milk composition and impact of the microbiome development remains largely unexplored in ruminants. This study investigated the role of mode of birth on changes to maternal transition and mature milk composition, and its subsequent effect on the abundance of microbial phyla in the calf gut. This work demonstrates that mode of delivery does affect the composition of early milk and has subsequent effects on the composition of the gut microbiome in neonatal calves. While significant differences were observed in milk composition of VD and CD cows more focus should be given to maternal environment since CD are rare in the livestock industry. Previous studies have demonstrated the impacts of maternal diet, age, parity, and season of calving on early milk composition. While this study begins to shed light on previously unknown relationships between mode of birth, early milk composition, and calf gut microbiome, more research is needed to fully comprehend the physiological mechanisms impacting milk composition due to the maternal environment. It is important to comprehend how biosynthetic pathways of lactose, milk proteins and fatty acids are being impacted by maternal environment in order to successfully develop better management programs with the ultimate goal of producing optimal quality colostrum. Additionally, determining the potential role of early milk components such as lactose and Oleic acid (C18:1) in the gut microbiota is important since, based on our results, they are responsible for most of the microbial phyla abundance fluctuations.

2. Tables

Table 2.1 Descriptive statistics by day of sampli**ng**

	Day 1			Day 3			Day 28		
Item	**Mean ± SE[a]**	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**
Component									
True Protein, %	8.42 ± 0.68	4.12	19.4	5.61 ± 0.42	3.37	13.68	3.25 ± 0.06	2.59	4.09
Fat, %	3.54 ± 0.29	0.76	7.89	5.18 ± 0.38	1.74	13.23	4.66 ± 0.47	1.34	11.72
SNF[b]	12.94 ± 0.59	9.18	22.36	10.26 ± 0.35	8.31	17.46	9.00 ± 0.07	8.16	9.82
Lactose	3.65 ± 0.10	1.89	4.50	3.89 ± 0.08	2.69	4.55	4.89 ± 0.04	4.28	5.25
SCC[c], Log_{10}	2.95 ± 0.09	2.07	4.30	1.60 ± 1.02	1.20	2.18	2.39 ± 1.02	1.58	3.89
Urea, mg/dl	17.60 ± 1.93	2.95	53.90	9.86 ± 1.22	2.80	32.65	7.30 ± 0.62	0.96	16.6
Fatty Acid g/100 g TFA[l]									
C14:0	.	.	.	16.65 ± 1.06	11.01	47.02	15.44 ± 0.64	9.86	27.08
C16:0	.	.	.	48.88 ± 3.66	33.23	149.64	36.71 ± 1.25	27.72	57.12
C18:0	.	.	.	8.14 ± 0.66	0.21	14.28	12.11 ± 0.38	8.38	16.76
C18:1	.	.	.	27.96 ± 1.36	0.39	37.48	30.29 ± 1.47	19.73	49.17
LCFA[d]	.	.	.	27. 78 ±1.82	0.21	42.80	32.33 ± 1.78	8.56	48.32
MCFA[e]	.	.	.	77.47 ± 4.86	52.51	176.92	44.60 ± 2.73	8.24	69.99
MUFA[f]	.	.	.	30.07 ± 1.41	0.39	46.26	33.54 ± 1.75	18.62	56.34
PUFA[g]	.	.	.	6.19 ± 0.37	2.07	12.38	7.94 ± 0.62	3.12	20.06
SFA[h]	.	.	.	78.28 ± 1.72	68.14	125.31	70.01 ± 1.36	55.92	82.11
TFA[i]	.	.	.	0.00 ± 0.00	0.00	0.00	0.00 ± 0.00	0.00	0.00
TUFA[j]	.	.	.	26.85 ± 1.68	0.39	39.88	30.67 ± 1.47	13.29	44.38
SCFA[k]	.	.	.	11.21 ± 0.30	5.07	13.49	9.56 ± 0.51	1.85	13.49

[a]SE = Standard error, [b]SNF =Solid non-fats, [c]SCC= somatic cell count, [d]LCFA = Long chain fatty acid, [e]MCFA = Medium chain fatty acid, [f]MUFA = Monounsaturated fatty acid, [g]PUFA = Polyunsaturated fatty acid, [h]SFA = Saturated fatty acid, [i]TFA = Trans fatty acid, [j]TUFA = Trans unsaturated fatty acid, [k]SCFA = Short chain fatty acid, [l]TFA = Total fatty acid.

Table 2.2: Mode of birth (i.e., vaginal versus cesarean delivery) regressed on composition of transition and mature milk by sampling day.

	Transition milk – d 1		Mature milk – d 3		Mature milk – d 28	
Item	**β[a] ± SE[b]**	***P*-value**	**β ± SE**	***P*-value**	**β ± SE**	***P*-value**
True Protein, %	0.09 ± 0.02	0.001	0.01 ± 0.01	0.001	0.01 ± 0.01	0.068
Fat, %	-0.06 ± 0.06	0.344	2.08 ± 0.16	0.205	0.30 ± 0.24	0.214
SNF[c], %	0.01 ± 0.00	0.001	0.00 ± 0.00	0.007	0.00 ± 0.00	0.442
Lactose, %	26.5 ± 6.93	< 0.001	16.1 ± 7.29	0.033	10.6 ± 6.45	0.111
SCC[d], cells/mL	0.03 ± 0.21	0.874	0.01 ± 0.02	0.705	$-1.2e^{-4}$ ± 0.04	0.997
Urea, mg/dL	-0.44 ± 0.09	< 0.001	-1.05 ± 0.31	0.001	-0.22 ± -0.76	0.453

[a] Regression coefficient of vaginal birth when compared to cesarean section.
[b]Standard Error (SE) of the estimate
[c]Solid Non-Fats abbreviated SNF
[d]Somatic Cell Count abbreviated SCC

Table 2.3: Mode of birth (i.e., vaginal versus cesarean delivery) regressed on mature milk fatty acid profile by day.

	Mature milk - d 3		Mature milk – d 28	
Item, g/100 g TFA[k]	**β[a] ± SE[b]**	***P*-value**	**β ± SE**	***P*-value**
C14:0	0.002 ± 0.001	0.029	0.003 ± 0.001	0.085
C16:0	0.00 ± 0.00	0.027	0.00 ± 0.00	0.335
C18:0	12.8 ± 4.81	0.011	0.25 ± 4.88	0.959
C18:1	5.58 ± 2.72	0.047	5.95 ± 3.38	0.089
LCFA[c]	31.1 ± 8.41	< 0.001	8.98 ± 12.6	0.483
MCFA[d]	0.03 ± 0.007	< 0.001	0.03 ± 0.02	0.297
MUFA[e]	8.48 ± 7.92	0.292	8.78 ± 12.8	0.499
PUFA[f]	-0.09 ± 0.04	0.051	-0.05 ± 0.07	0.466
SFA[g]	0.00 ± 0.00	0.144	0.00 ± 0.00	0.461
TFA[h]	-0.03 ± 0.06	0.592	0.17 ± 0.16	0.282
TUFA[i]	29.6 ± 7.55	< 0.001	16.5 ± 10.0	0.110
SCFA[j]	54.20 ± 24.50	0.033	0.03 ± 0.18	0.857

[a] Regression coefficient of vaginal birth when compared to cesarean section.
[b]Standard Error (SE) of the estimate
[c]LCFA = Long chain fatty acid
[d]MCFA = Medium chain fatty acid
[e]MUFA = Monounsaturated fatty acid
[f]PUFA = Polyunsaturated fatty acid
[g]SFA = Saturated fatty acid
[h]TFA = Trans fatty acid
[i]TUFA = Trans unsaturated fatty acid
[j]SCFA = Short chain fatty acid
[k]TFA = Total fatty acid

Table 2.4: Milk composition and fatty acid profile regressed on rumen Actinobacteria abundance over time.

	Transition milk - d 1		Mature milk - d 3		Mature milk – d 28	
Item	**β ± SE**	***P* – value**	**β ± SE**	***P* - value**	**β ± SE**	***P* - value**
Component						
True Protein, %	-2.37± 1.03	0.033	0.51 ± 2.02	0.801	-0.94 ± 1.38	0.504
SNF[c], %	19.10 ± 9.06	0.049	-81.30 ± 75.2	0.292	106.00 ± 196.00	0.596
Lactose, %	0.00 ± 0.00	0.030	$-3.0e^{-4}$ ± 0.00	0.753	0.00 ± 0.00	0.704
Urea, mg/dl	0.02 ± 0.04	0.565	-0.03 ± 0.01	0.015	-0.02 ± 0.01	0.208
Fatty Acid, g/100 g TFA[h]						
C14:0	.	.	4.66 ± 10.7	0.669	-4.98 ± 10.00	0.628
C16:0	.	.	250 ± 322	0.448	-286.00 ± 328.00	0.398
C18:0	.	.	$-1.8e^{-4}$ ± 0.00	0.920	0.00 ±0.00	0.943
C18:1	.	.	0.00 ± 0.00	0.006	0.00 ± 0.00	0.653
LCFA[d]	.	.	$-9.5e^{-4}$ ± 0.00	0.608	0.00 ± 0.00	0.741
MCFA[e]	.	.	0.18 ± 0.85	0.830	-0.33 ± 0.54	0.547
TUFA[f]	.	.	-0.002 ± 0.00	0.231	0.00 ± 0.00	0.467
SCFA[g]	.	.	$-1.1e^{-4}$ ± 0.00	0.457	0.00 ± 0.00	0.876

[a] Estimate indicating a change in the response variable.
[b]Standard Error (SE) of the estimate
[c]Solid Non-Fats abbreviated SNF
[d]LCFA = Long chain fatty acid
[e]MCFA = Medium chain fatty acid
[f]TUFA = Trans unsaturated fatty acid
[g]SCFA = Short chain fatty acid
[h]TFA = Total fatty acid

Table 2.5: Milk composition and fatty acid profile regressed on rumen Bacteroidetes abundance over time

	Transition milk - d 1		Mature milk - d 3		Mature milk - d 28	
Item	**β ± SE**	***P* - value**	**β ± SE**	***P* - value**	**β ± SE**	***P* - value**
Component						
True Protein, %	0.03 ± 0.37	0.936	3.05 ± 6.13	0.623	1.01 ± 2.12	0.638
SNF[c], %	-0.67 ± 3.30	0.840	-197.00 ± 228.00	0.395	-61.10 ± 301.00	0.841
Lactose, %	0.00 ± 0.00	0.598	$-2.6e^{-4}$ ± 0.00	0.927	0.00 ± 0.00	0.853
Urea, mg/dl	0.00 ± 0.01	0.966	0.02 ± 0.04	0.619	0.03 ± 0.02	0.297
Fatty Acid, g/100 TFA[h]						
C14:0	.	.	3.94 ± 28.00	0.889	-4.98 ± 10.00	0.071
C16:0	.	.	-916.00± 844.00	0.293	-286.00 ± 328.00	0.190
C18:0	.	.	0.00 ± 0.00	0.069	0.00 ± 0.00	0.999
C18:1	.	.	-0.01 ± 0.00	0.004	0.00 ± 0.00	0.790
LCFA[d]	.	.	0.00 ± 0.00	0.541	0.00 ± 0.00	0.500
MCFA[e]	.	.	4.12 ± 2.22	0.081	-0.33 ± 0.54	0.432
TUFA[f]	.	.	0.00 ± 0.00	0.809	0.00 ± 0.00	0.147
SCFA[g]	.	.	0.00 ± 0.00	0.930	0.00 ± 0.00	0.527

[a]Estimate indicating a change in the response variable.
[b]Standard Error (SE) of the estimate
[c]Solid Non-Fats abbreviated SNF
[d]LCFA = Long chain fatty acid
[e]MCFA = Medium chain fatty acid
[f]TUFA = Trans unsaturated fatty acid
[g]SCFA = Short chain fatty acid
[h]TFA = Total fatty acid

Table 2.6: Milk composition and fatty acid profile regressed on rumen Firmicutes abundance over time

	Transition milk - d 1		Mature milk - d 3		Mature milk – d 28	
Item	**β ± SE**	***P* - value**	**β ± SE**	***P* - value**	**β ± SE**	***P* - value**
Component						
True Protein, %	38.00 ± 19.00	0.062	4.33 ± 29.50	0.884	7.32 ± 8.68	0.410
SNF[c], %	-278 ± 167	0.115	310.00 ±1099.00	0.780	39.60± 1229.00	0.974
Lactose, %	-0.04 ± 0.01	0.009	0.00 ± 0.01	0.670	$-7.9e^{-4}$ ± 0.01	0.940
Urea, mg/dl	-0.56 ± 0.29	0.470	-0.18 ± 0.21	0.388	-0.14 ± 0.10	0.180
Fatty Acid, g/100 TFA[h]						
C14:0	.	.	40.10 ± 156.00	0.800	36.20 ± 68.20	0.603
C16:0	.	.	9466.00 ± 4694.00	0.059	-1124.00 ± 2228.00	0.622
C18:0	.	.	-0.02 ± 0.02	0.326	0.01 ± 0.01	0.439
C18:1	.	.	0.06 ± 0.02	0.034	0.00 ± 0.02	0.987
LCFA[d]	.	.	-0.02 ± 0.02	0.375	-0.01 ± 0.01	0.591
MCFA[e]	.	.	-6.99 ± 12.30	0.579	-0.81 ± 3.68	0.829
TUFA[f]	.	.	0.00 ± 0.02	0.798	0.01 ± 0.02	0.685
SCFA[g]	.	.	0.00 ± 0.00	0.688	0.00 ± 0.00	0.919

[a] Estimate indicating a change in the response variable.
[b]Standard Error (SE) of the estimate
[c]Solid Non-Fats abbreviated SNF
[d]LCFA = Long chain fatty acid
[e]MCFA = Medium chain fatty acid
[f]TUFA = Trans unsaturated fatty acid
[g]SCFA = Short chain fatty acid
[h]TFA = Total fatty acid

Table 2.7: Milk composition and fatty acid profile regressed on rumen Proteobacteria abundance over time

	Transition milk - d 1		Mature milk - d 3		Mature milk - d 28	
Item	**β ± SE[b]**	***P* - value**	**β ± SE**	***P* - value**	**β ± SE**	***P* - value**
Component						
True Protein, %	-26.6 ± 9.64	0.013	6.01 ± 23.30	0.798	-23.40 ± 19.20	0.239
SNF[c], %	213.00 ± 84.40	0.021	-255.00 ± 901.00	0.779	1923.00 ± 2670.00	0.480
Lactose, %	0.02 ± 0.01	0.002	0.00 ± 0.01	0.876	0.02 ± 0.02	0.300
Urea, mg/dl	0.11 ± 0.39	0.775	-0.05± 0.17	0.734	-0.01 ± 0.23	0.964
Fatty Acid, g/100 g TFA[h]						
C14:0	.	.	-169.00 ± 137.00	0.232	-158.00 ± 126.00	0.232
C16:0	.	.	-1123.00 ± 4125.00	0.788	-412.00 ± 4139.00	0.922
C18:0	.	.	0.01 ± 0.02	0.589	-0.02 ± 0.03	0.428
C18:1	.	.	0.02 ± 0.02	0.272	0.02 ± 0.05	0.699
LCFA[d]	.	.	0.00 ± 0.02	0.860	0.01 ± 0.03	0.587
MCFA[e]	.	.	-18.60 ± 10.80	0.104	1.30 ± 6.83	0.851
TUFA[f]	.	.	0.01 ± 0.02	0.477	0.00 ± 0.05	0.940
SCFA[g]	.	.	0.001± 0.00	0.348	0.00 ± 0.00	0.570

[a] Estimate indicating a change in the response variable.
[b]Standard Error (SE) of the estimate
[c]Solid Non-Fats abbreviated SNF
[d]LCFA = Long chain fatty acid
[e]MCFA = Medium chain fatty acid
[f]TUFA = Trans unsaturated fatty acid
[g]SCFA = Short chain fatty acid
[h]TFA = Total fatty acid

REFERENCES

Ahn, S.-J., Wen, Z. T., & Burne, R. A. (2007). Effects of Oxygen on Virulence Traits of Streptococcus mutans. *Journal of Bacteriology, 189*(23), 8519-8527. doi: 10.1128/JB.01180-07

Akers, R. M. (Ed.) (2002). *Chapter 4: Milk Component Biosynthesis. Lactation and the Mammary* (First ed.).

Alipour, M. J., Jalanka, J., Pessa-Morikawa, T., Kokkonen, T., Satokari, R., Hynonen, U., . . . Niku, M. (2018). The composition of the perinatal intestinal microbiota in cattle. *Sci Rep, 8*, 10437. doi:doi:10.1038/s41598-018-28733-y

Argenio, D., & F, S. (2015). The role of the gut microbiome in the healthy adults status. *Clinica Chimic Acta, 451*, 97-102. doi:10.1016/j.cca.2015.01.003

Bagnicka, E., Winnicka, A., Jozwik, A., Rzewuska, M., Strzalkowska, N., Kosciuczuk, E., . . . Krzyzewski, J. (2011). Relationship between somatic cell count and bacterial pathogens in goat milk. *Small Ruminant Research, 100*, 72-77. doi:10.1016/j.smallrumres.2011.04.014

Binda, C., Lopetuso, L. R., Rizzatti, G., Gibiino, G., Cennamo, V., & Gasbarrini, A. (2018). Actinobacteria: A relevant minority for the maintenance of gut homeostasis. *International Journal of Gastroenterology and Heptology, 50*(5), 421-428. doi:10.1016/j.dld.2018.02.012

Bionaz, M., Hurley, W., & Loor, J. (2012). Milk Protein Synthesis in the Lactating Mammary Gland: Insight from Transcriptomic Analyses. *IntechOpen Milk Protein*. doi:10.5772/46054

Birg, A., Ritz, N. L., & Lin, H. C. (2000). The Unknown Effect of Antibiotic-Induced Dysbiosis on the Gut Microbiota. In J. Faintuch & S. Faintuch (Eds.), *Microbiome and the*

Metabolome in Diagnosis, Therapy, and other Strategic Applications (pp. 195-200). Academic Press.

Bokulich, N. A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H., . . . Blaser, M. J. (2016). Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Science Translational Medicine, 8*(343), 82. doi:10.1126/scitranslmed.aad7121

Collomb, M., Bisig, W., Butikofer, U., Sieber, R., Bregy, M., & Etter, L. (2008). Seasonal variation in the fatty acid composition of milk supplied to diaries in the mountain regions of Switzerland. *Dairy Sci. Technol*. 88, 631-647. doi:10.1051/dst:2008029

Cunningham, H. C., Austin, K. J., & Cammack, K. M. (2018). Influence of maternal factors on the rumen microbiome and subsequent host performance. *Translational Animal Science, 2*(1), S101-S105. doi:doi.org/10.1093/tas/txy058

Cunningham, H. C., Austin, K. J., Powell, S. R., Carpenter, K. T., & Cammack, K. M. (2018). Potential response of the rumen microbiome to mode of delivery from birth through weaning. *Translational Animal Science, 2*(1), S35-S38. doi:10.1093/tas/txy029

Dizdar, E. A., Sari, F. N., Degirmencioglu, H., Canpolat, F. E., Oguz, S. S., Uras, N., & Dilmen, U. (2013). Effect of mode of delivery on macronutrient content of breast milk. *The Journal of Maternal-Fetal & Neonatal Medicine, 27*(11), 1099-1102. doi:10.3109/14767058.2013.850486

Dunn, A., Ashfield, A., Earley, B., Welsh, M., Gordon A., & Morrison S.J. (2017). Evaluation of factors associated with immunoglobulin G, fat, protein, and lactose concentration in bovine colostrum and colostrum management practices in grassland based dairy systems in Northern Ireland. J. Dairy. Sci. 100(3), 2068-2079. doi: 10.3168/jds.2016-11724

Dunn, A. B., Jordan, S., Baker, B. J., & Carlson, N. S. (2017). The maternal infant microbiome: Considerations for labor and birth. *MCN Am J Matern Child Nurs, 42*(6), 318-325. doi:10.1097/NMC.0000000000000373

Elsen, L. W. J. V. d., Garssen, J., Burcelin, R., & Verhasselt, V. (2019). Shaping the gut microbiota by breastfeeding: The gateway to allergy prevention? *Front. Pediatr., 7*, 47. doi:10.3389/fped.2019.00047

Fahmy, M. H. (1972). Comparative study of colostrum and milk composition of seven breeds of swine. *Canadian Journal of Animal Science, 52*(4), 621-627. doi:10.4141/cjas72-073

FOSS. (2017). MilkoScan FT+/FT 6000 Fatty Acid Prediction models. Application Note 0064/Rev 5. (37):1-37.

Hanuš, O., Samková, E., Křížová, L., Hasoňová, L., & Kala, R. (2018). Role of Fatty Acids in Milk Fat and the Influence of Selected Factors on Their Variability-A Review. *Molecules (Basel, Switzerland), 23*(7), 1636. https://doi.org/10.3390/molecules23071636

Krajmalnik-Brown, R., Ilhan, Z. E., Kang, D. W., & DiBaise, J. K. (2012). Effects of gut microbes on the nutrient absorption and energy regulation. *Nutr Clin Pract, 27*(2), 201-214. doi:10.1177/0884533611436116

Langer, P. (2009). Differences in the compostion of colostrum and milk in eutherians reflect differences in immunoglobulin transfer. *Journal of Mammology, 90*(2), 332-339. doi:10.1644/08-MAMM-A-071.1

Lodge-Ivey, S. L., Brown-Silva, J., & Horvath, M. B. (2009). Technical note: Bacterial diveristy and fermentation end products in rumen fluid samples collected vi oral lavage or rumen cannula. *J Anim Sci, 87*(7), 2333-2337. doi:10.2527/jas.2008-1472

Malmuthuge, N., Griebel, P. J., & Guan, L. L. (2015). The gut microbiome and its potential role in the development and function of newborn calves gastrointestinal tract. *Front Vet Sci, 2*(36). doi:10.3389/fvets.2015.00036

Malmuthuge, N., Liang, G., & Guan, L. L. (2019). Regulation of the rumen development in neonatal ruminants through microbial metagenomes and host transcriptomes. *Genome Biology, 20*, 172. doi:10.1186/s13059-019-1786-0

McGrath, B. A., Fox, P. F., McSweeney, P. L., & Kelly, A. L. (2016). Composition and properties of bovine colostrum: a review. *Dairy Sci & Technol, 96*, 133-158. doi:10.1007/s13594-0258-x

Osorio, J. S., Lohakare, J., & Bionaz, M. (2016). Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation. *Physio Genomics, 48*, 231-256. doi:10.1152/physiogenomics.00016.2015

Powell, S., Cunningham, H. C., Austin, K. J., & Cammack, K. M. (2018). Maternal influences on beef calf rumen microbiome in the first 4 wk of life. *Trans Anim Sci, 2*(1), S96. doi:10.1093/tas/txy077

Puppel, K., Gołębiewski, M., Grodkowski, G., Slósarz, J., Kunowska-Slósarz, M., Solarczyk, P., Łukasiewicz, M., Balcerak, M., & Przysucha, T. (2019). Composition and Factors Affecting Quality of Bovine Colostrum: A Review. *Animals : an open access journal from MDPI, 9*(12), 1070. https://doi.org/10.3390/ani9121070

Rizzatti, G., Lopetuso, L. R., Gibiino, G., Binda, C., & Gasbarrini, A. (2017). Proteobacteria: A common factor in human disease. *BioMed Research International, 2017*. doi:10.1155/2017/9351507

Rudolph, M. C., Russell, T. D., Webb, P., Neville, M. C., & Anderson, S. M. (2011). Prolactin-mediated regulation of lipid biosynthesis genes in vivo in the lactating mammary epithelial cell. *American journal of physiology. Endocrinology and metabolism*, *300*(6), E1059–E1068. https://doi.org/10.1152/ajpendo.00083.2011

Sheridan, O., Martin, P. J. C., Lawley, T. D., Browne, H. P., Harris, H., Donadille, B., & Flint, A. J. (2016). Polysaccharide utilization loci and nutritional specialization in a dominant group of butyrte-producing human colonic Firmicutes. *Microbial Genomics, 2*(2). doi:10.1099/mgen.0.000043

Sinanoglou, V. J., Cavouras, D., & Boutsikou, T. (2017). Factors affecting human colostrum fatty acid profile: A case study. *PLos One, 12*(4), e0175817. doi:10.1371/journal.pone.0175817

Waters, S. B., & Rillema, J. A. (1988). Effect of prolactin on enzymes of lipid biosynthesis in mammary gland explants. *Am J Physiol*, *255*(18), E567-E571.

Wexler, H. M. (2007). Bacteroidetes: The good, the bad, and the nitty-gritty. *Clinical Microbiology Reviews, 20*(4), 593-621. doi:10.1128/CMR.00008-07

Yu, Z., & Morrison, M. (2004). Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques, 36*(5), 808-812. doi:10.2144/04365ST04

Zhu, J., & Luo, J. (2017). The synthesis of milk medium-chain fatty acids in mammary gland. *IntechOpen*. doi:10.5772/67694

CHAPTER 3: GENETIC PARAMETERS OF BRIX SCORE AND COLOSTRUM WEIGHT

Abstract

The objectives of this research were to 1) establish phenotypic relationships between colostrum composition traits, milk production traits, and calf health, 2) determine impact of breed and season on colostrum production and 3)) elucidate the genetic parameters (i.e. heritability, genotypic, and phenotypic correlations) among colostrum production and milk production. Colostrum samples were collected from three dairy farms over the course of one year. Jersey (n = 289) and Holstein (n = 250) primi-parous (n = 166) and multi-parous (n = 407) cows were used in this study. First milking colostrum samples were collected within 12 hours post-partum, whereby, cows were completely milked to determine Brix scores and colostrum volume. Colostrum samples for Jersey cows were sent to a commercial milk laboratory for composition quantification, and lactoferrin concentrations were determined via ELISA to establish relationship of colostrum components with quality traits and asses if there is a relationship between colostrum composition and calf incidence of disease. Calf blood samples were collected 24-72 h post-partum and total serum protein quantified to assess passive immunity transfer and determine relationship with incidence of disease. A PROC Mixed with LSMEANS was performed in SAS to determine relationships between colostrum components, test-day milk components, and quality traits for season, breed, and the interaction between season and breed. Furthermore, a Pearson correlation was used to determine phenotypic relationships among colostrum composition traits and colostrum quality traits. A PROC Mixed with LSMEANS was used to determine relationships of calf health with season, freshness of colostrum, total serum protein, volume fed, brix of colostrum fed, colostrum source and colostrum components for Jersey calves. Results for test-day milk, protein, fat, and somatic cell count (**SCS**) for each cow was obtained from the USDA Animal Genomic and Improvement Laboratory (**AGIL**). Breeds

were evaluated separately by BLUPF90 using a single-trait repeatability animal model to estimate heritability and repeatability. Phenotypic analysis for Holstein and Jersey cows indicated significant interaction of breed and season for colostrum volume but not Brix score. Season was a significant factor affecting colostrum volume, lactose, lactoferrin, and test day (i.e. first test day after calving greater than 14 days in milk) milk, fat, and protein. Additionally, there were significant differences ($P < 0.001$) for colostrum volume and all test-day components between the breeds. There was a strong phenotypic correlation between true protein and Brix ($r_p = 0.86$). Additionally, calf incidence of respiratory illness was impacted by season (marginally), total serum protein levels (**TSP**), and colostrum components such as true protein and solid non-fats (**SNF**). Heritability estimates for Brix and colostrum were moderate ($h^2 = 0.15$ to 0.47) for Holstein and Jersey cows. There was a strong genetic correlation between Brix score with colostrum volume (-0.98) in Jersey cows, and colostrum volume and test-day milk yield (0.96) for Holstein cows. In conclusion, differences in quality traits, colostrum composition, and test day components by season and breed were observed. Calf incidence of respiratory disease was impacted by protein content in colostrum and levels of total serum protein which present the importance of having optimal colostrum quality. Additionally, colostrum quality and volume are heritable traits, which suggests that genetic improvement of these traits is possible. However, deviations of genetic correlations between breeds for colostrum quality and volume with test-day milk composition suggests that innate differences exist between Holstein and Jersey cows for colostrum and milk production.

INTRODUCTION

Consumption of 3.78 L of colostrum with immunoglobulins (IgG) > 50 mg/mL within the first 6 hrs post-partum, due to calf gut closure which gradually decreases IgG absorption within 24 hr post-partum, is imperative for the health of a newborn calf (Godden, 2019). Not only does it contain nutrients optimized for calf gut maturity but is critical for the development of the calf's immune system (Yang et al., 2015). Providing calves with sufficient high-quality colostrum (4 L in first feeding) decreased rates of calf mortality by 50% when compared to calves fed 2 L of high-quality colostrum (Robison et al., 1988). Pre-weaned heifer calf deaths account for > $100 million in annual losses in the U.S., which does not account for preventative and treatment costs related to illnesses (USDA-APHIS, 1992). Additionally, costs associated with lifetime profitability losses and increased feed inputs related to poor growth of sick calves are not included in this estimate. A great percentage (> 60%) of maternal colostrum in the US is considered inadequate or of low quality (Morrill et al., 2012). Additionally, colostrum volume in dairy cows is variable but investigators report that the vast majority of cows produce below optimal (< 8.5 kg) colostrum (Pritchett et al, 1991). Biological factors such as breed, dam age, parity, season of calving, diet, and dry-period length are thought to influence colostrum quality and quantity. A major challenge for colostrum production is that colostrum quality and volume tend to be inversely related. Therefore, being able to improve colostrum quality (i.e. Brix and volume) and elucidate the impacts of biological factors on quality traits can mitigate the negative economic impacts that come with incidence of illness and mortality in calves and enhance calf health.

Much research has been conducted on the impact of colostrum IgG, one of the most important proteins in colostrum, on calf health. However, not much is understood about the role

of other colostrum components on calf health or long-term milk production. True protein in colostrum measures the overall protein content of colostrum. This is important because 80% of true protein in colostrum is immunoglobulins which are important for passive immunity for the calf (McGrath et al., 2016). Additionally, solids non-fats (**SNF**) account for the components in colostrum that are not fat, such as casein, whey proteins, lactose and minerals. The role of SNF in calf health is not completely understood but it is thought to bring nutritional value to the neonate as well as some health benefits linked to the protein lactoferrin (Yamauchi et al., 2006). Lactoferrin is one example of a non-specific mammary protein that reduces availability of iron to bacteria in the gut, thereby diminishing bacterial growth of potentially harmful bacterial in the calf gut. Additionally, lactoferrin has been associated with improved gain-to-feed ratios, increased average daily gains, improved fecal scores, and reduced morbidity in pre-weaned calves (Robblee et al., 2003). Furthermore, lactose can influence colostrum volume which could impact the supply of colostrum to the neonatal calf (Boland, 2011). Lactose also increases the concentration of Bifidobacteria in the gut, which is beneficial for healthy gut development, giving it prebiotic properties (Dahl, 2012). Fat is another important component in colostrum, while it has not been directly linked with calf health it mainly provides caloric intake. In addition, studies have shown that fat is associated with increased metabolism of brown adipose tissue that help calves thermoregulate and maintain their body temperatures (Kehoe, 2006). Urea is a breakdown product of protein which is also found in colostrum. There have been no nutritional or health benefits associated with colostral urea to date. In fact, calves at this age cannot consume large amounts of urea. However, urea could still be used as an indirect measure of how the dam is managed (i.e. diet, dry period…) which can heavily influence colostrum quality (Nowak et al., 2012).

Maternal factors such as age, parity, season of calving, and diet can also impact quality of colostrum which can ultimately impact calf health (Godden, 2019). Older animals with 2 or more parity have increased concentration of immunoglobulins in colostrum when compared to first parity animals which contribute to colostrum quality (Godden, 2019). Additionally, season impacts quality of colostrum with fall having the greatest immunoglobulin content when compared to other seasons (Gulliksen et al., 2008). Immunoglobulin concentration (IgG) and volume of colostrum fed influences success of passive immunity in calves; therefore, maternal environment needs to be carefully regulated to promote better quality colostrum and in turn healthier calves (McGrath et al., 2016). There are also breed differences that may play a role in determining how breeding programs and selection indexes are developed. Both Holstein and Jersey cows have compositional differences of colostrum with Jersey colostrum tending to have greater levels of lactoferrin, immunoglobulins, alpha-lactalbumin and fat (Guy et al., 1994; Boland, 2011; Rocha et al., 2014). Therefore, breed differences impact on colostrum quality traits need to further be understood in order to determine a specific animal's genetic value in a herd.

Because individuals vary in their colostrum production and there appears to be an inherent difference among breeds, it is critical that we elucidate the environmental and genetic mechanisms that regulate colostrum quality (i.e Brix and volume), composition, and output. By doing so, we will be able to develop a genetic selection index for colostrum production that allows for optimal colostrum quality and volume while not negatively impacting mature milk production traits. We hypothesized that colostrum composition and production differ among breeds and by season and that individual components influence calf health within 2 m post-partum. Additionally, we hypothesized that colostrum quality traits, Brix score and volume, are

heritable. This study has three objectives: 1) establish phenotypic relationships between colostrum composition traits, milk production traits, and calf health, 2) determine impact of breed and season on colostrum production, and 3) elucidate the genetic parameters (i.e. heritability, genotypic, and phenotypic correlations) among colostrum production and milk production. Determining heritability and correlation values would allow for superior animals to be identified and be genetically selected for their quality traits. Elucidating the phenotypic and genetic interrelationships of colostrum composition and output on calf health and long-term performance will enable researchers to develop genetic selection tools to improve colostrum production.

MATERIALS AND METHODS

Animals and Management. All animal procedures were approved by the Virginia Polytechnic Institute and State University Animal Care and Use Committee (# 17-249 & # 18-071). Multi-parous (n = 407) and primi-parous (n = 166) Holstein cows (n = 250) and Jersey cows (n = 289) were measured for colostrum volume, colostrum quality (i.e. Brix and volume) and colostrum composition using first milking colostrum samples collected within 12 hrs post-calving. Data from Holstein cows were collected from the Virginia Tech Dairy Complex (Kentland, VA; Farm 2). Data from Jersey cows came from the Virginia Tech Dairy Complex and two additional farms (Farm 1 and 3) within 400 km of each other. Herd size ranged from 200 to 500 cows which includes lactating, dry, and heifer cows. The study was conducted over the course of one year (2018 to 2019). Average ages of cows were 5.90, 6.6, and 8.90 yrs for farm 1, 2, and 3, respectively. Farm 1 and 3 had a dry period length of 50-60 d, however, some cows had dry periods under 40 days. Farm 2 had a larger range with a dry period from 14-90 d. At farm 1, far off cows were fed grass hay and corn silage while close up cows were fed dry cow

grain and limited silage. Farm 2 fed 65% roughage, 25% concentrate, and 9% byproduct for far off cows while close up cows were fed 73% roughage and 27% concentrate. On farms 1 and 2, cows are housed on pasture or free stall barns while farm 3 housed their cows on a 50-acre pasture with no free stalls. At farm 3 cows were fed pelleted feed (about 0.90 kg pounds per day per cow) along with some dry grass hay. Diets for far off and close up cows did not differ for farm 3.

After parturition, calves were separated from dam and not allowed to suckle. Cows were milked within 24 hrs post-partum and a bucket was used to collect colostrum and then weighed on an analog scale to determine colostrum volume. A Brix score was determined using a calibrated 0-32% Brix refractometer tool set (Vee Gee Scientific, Vernon Hills, IL); whereby, 500 μL of colostrum was placed on the sample plate. For Jersey cows two, 50 mL colostrum samples of colostrum were collected in conical tubes: one for Dairy Herd Improvement Association (**DHIA**) with preservative and another for lactoferrin analyses via ELISA. Samples for DHIA were measured for colostrum components (i.e. fat, true protein, solid non-fats, SCS, urea) while the remaining sample was stored in a conventional freezer on the farm until further processing. All sample collection, weighing, and Brix determination was performed on-farm by trained staff.

Dam age at calving, calving year (2018 or 2019), parity (primiparous vs multiparous), 3-month season of calving (March-May; n = 203, June-August; n = 192, September-November; n =97, and December-February; n = 114) were recorded via PC Dart (DRMS-NCSU, Raleigh, NC). Due to missing records (i.e. maternal records, birth weight, feeding records) 67 dams were dropped from study. Test-day compositional traits (i.e. first test day after calving greater than 14 days in milk) values of milk were provided by USDA-AGIL (Beltsville, MD, USA).

Jersey and Holstein heifer calf blood samples were collected from jugular vein using 10 mL glass vacutainer tubes (BD Vacutainer®, New Jersey) with no additives within 72 h post-colostrum consumption. Samples from farm 1 were centrifuged (centrifuge provided by Virginia Tech) and stored on site. Farm 2 samples were then transported to Cockrum laboratory and farm 3 samples were transported to Kingdom Animal Hospital (Clear Brooke, VA) for centrifugation and storage. Blood samples were transported on ice and stored overnight at -4° C. After, samples were centrifuged for 20 minutes at 1200 × g at 4° C and then aliquoted into three, 1.5 mL Eppendorf tubes and stored at -80° C until analyses. Total serum protein was quantified using a 3 in 1 Scale Clinical Refractometer (Danoplus, Hong Kong, China) as an indirect measurement of serum IgG to determine if calves achieved passive immunity transfer. Additionally, farm staff were instructed to record calf birth weight, amount of first and second colostrum feeding, source of colostrum fed, total colostrum fed, and Brix score of colostrum fed. Incidence of scours or respiratory disease within the first 2 m post-partum was obtained from PC Dart (DRMS-NCSU, Raleigh, NC). For DHIA, samples were analyzed with the Bentley 2000 mid-infrared (Bentley Instruments, Inc, Chaska, MN) to determine protein, fat, lactose, SCS, and urea concentrations. Concentration of components are predicted from the spectrum of light reflected off chemical bonds in the milk sample.

Lactoferrin Quantification. Lactoferrin was quantified from Jersey colostrum samples using a Bovine Lactoferrin Enzyme-Linked Immunosorbent Assay Quantitation set, E10-126 (Bethyl Laboratories, Inc., Montgomery, TX). A 96-well plate was coated with a mix of 10 mL ELISA Coating Buffer (0.05 M Carbonate-Bicarbonate, pH 9.6) and 10 μL of Affinity purified Goat anti-Bovine Lactoferrin Coating Antibody (A10-126A-7, Bethyl Laboratories), and incubated at room temperature for 60 min. During incubation, colostrum samples and standards

were prepared at initial 1:2000 dilution (5 µL colostrum sample and calibrator (RC10-126-10) into 10 mL of Phosphate Buffered Saline; **PBS**). Serial dilutions were performed taking 500 µL of initial dilution into subsequent Eppendorf tubes filled with 500 µL of PBS (dilution factors ranged from initial 1:2000 to final 1:128,000). Next, the 96-well plate was washed 5 × with ELISA wash solution (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0) and then wells filled with 200 µL of ELISA Blocking solution (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0) followed by a 30 min incubation period at room temperature. After incubation, plates were washed 5 × followed by samples and standards loaded onto plates in duplicate and incubated for another 60 min at room temperature. Next, plates were washed 5 × and then each well was loaded with 100 µL of HRP Conjugated Goat anti-Bovine Lactoferrin detection antibody (Dilution 1:200,000; A10-126P-32, Bethyl Laboratories) followed by a 60 min incubation period. After incubation, plates were washed 5 × followed by addition of 100 µL of Enzyme Substrate, TMB (Cat. No. E115, Bethyl Laboratories) to each well. Plates were then incubated in the dark for 5 min to minimize oversaturation. After final incubation, an additional 100 µL of ELISA stop solution (0.18 M H_2SO_4) was added to each well. An ELISA Plate reader (BioTek Instruments, Winooski, VT) was used for absorbance measurements (at 450 nm) and results from reading were input into MyAssays analysis software (MyAssays Ltd, Brighton, UK). A Four Parameter Logistic Curve was generated and samples with absorbance measures with an intra assay CV of < 10% and inter assay CV of < 15% were used to determine lactoferrin concentration.

Statistical Analysis

Descriptive statistics. The Mean procedure in SAS 9.4 was used to obtain descriptive statistics. For Holstein and Jersey cows, the mean, standard deviation, minimum and maximum

were calculated for colostrum Brix and volume, test-day milk, fat, protein, SCS, and dam age. Additionally, colostrum protein, fat, lactose, solids non-fat, SCS, urea, and lactoferrin descriptive statistics were also calculated for Jerseys. Calf records such as birth weight, TSP, and first colostrum feeding Brix, amount, source (i.e. dam, mix, other dam), freshness (i.e. fresh or frozen), and total amount fed also underwent the PROC Mean procedure (**Table 3.1 & 3.2**).

Phenotypic analyses. A PROC GLM was used to determine the relationship between Brix score and colostrum volume with main effects of season and breed. Additionally, a PROC Mixed was used to determine the relationship between colostrum Brix score and volume, colostrum components (JE only), and test day components with the main effects of 3-month season and breed. Adjusted colostrum means and standard errors for breed and season are reported using LSMEANS. Furthermore, the interaction between breed and season was tested for colostrum Brix and volume as well as test day components. Dame age, parity, and season of calving were used as covariates (**Table 3.3**).

Jersey colostrum components were quantified and a Pearson correlation in SAS 9.4 was done to determine significant relationships among colostrum compositional traits (i.e. true protein, fat, lactose, lactoferrin…) and colostrum quality traits (Brix score and volume).

A PROC Mixed was used to assess the environmental, colostrum, and management relationships with calf health. Fixed effects of season (i.e. Fall, Spring, Summer Winter), Brix of colostrum fed, TSP, and colostrum components and random effects freshness of colostrum fed (i.e. fresh or frozen), colostrum source (i.e. dam or mix), and volume of colostrum fed were evaluated against incidence of diarrhea and respiratory illness. Incidence of scours, respiratory illness, and colostrum freshness were input as binomial variables with animals that developed disease

represented with a 1 and those that did not fall ill a 0, and animals fed fresh colostrum represented by 1 and frozen a 0. (**Table 3.5**)

Prediction of (co)variance components and SNP effects

*Heritability estimates and repeatability***.** (Co)variance components were estimated for each phenotype (Brix score, colostrum weight, and test-day milk, fat, protein, and SCS yields) using the BLUPF90-family of programs (Misztal et al., 2014) with a single-trait repeatability animal model. Breeds were evaluated separately. The model used was:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z_a a} + \mathbf{Z_p p} + \mathbf{e}$$

where **y** is the 1-vector of phenotypes; β is a vector of fixed effects, which includes calving year (2018 or 2019), parity (1 or 2+), 3-month season of calving (February-May, June-August, September-November, and December-February), and age of the cow at the time of calving; **a** is a vector of random animal effects where $\mathbf{a} \sim N(0, \mathbf{A}\sigma^2_a)$ and **A** represents the numerator relationship matrix and σ^2_a the additive genetic variance; **p** is a vector of random permanent environment effects; **e** is a vector of random residual errors where $e \sim N(0, \mathbf{I}\sigma^2_e)$, where **I** represents an identity matrix and σ^2_e the residual error; and $\mathbf{X}$, $\mathbf{Z_a}$, and $\mathbf{Z_p}$ are corresponding design matrices relating phenotypes to levels of fixed and random effects. The Standard errors for additive genetic, permanent environmental and residual variance and covariance components were computed. This was done by taking the square root of diagonal elements of the inverse of the average information matrix.

*Pairwise genetic correlations***.** Genetic correlations of Brix score and colostrum volume with test-day milk, fat, protein, and SCS were estimated using a series of bivariate models. Correlations among the milk yield traits were not estimated due to the existence of an extensive

literature on that topic (e.g., Miller et al., 2004). Using the following equation, correlations were derived from co-variance component estimates:

$$r_{(\alpha,p)} = \frac{Cov_{(\alpha,p)}(X,Y)}{\sqrt{\sigma^2_{(\alpha,p)X} * \sigma^2_{(\alpha,p)Y}}}$$

The genetic correlation of Brix score with colostrum weight was estimated for both breeds using AI-REML as implemented in airemlf90 version 1.144. Genetic correlations of Brix score and colostrum weight with milk traits was estimated in a two-step process: restricted maximum likelihood as implemented in remlf90 version 1.84 was used to produce priors that were fed into airemlf90. When AI-REML was used with naïve priors there were problems with some variance estimates moving towards 0, which may be due to the limited size of the available data.

RESULTS

Descriptive statistics. Summary statistics are provided in **Table 3.1** for both Holstein and Jersey cows. Mean values for Brix were within normal range for Holstein cows (Negussie et al., 2013). Mean Brix score was 15% less than previous findings for Jersey cows (Gavin et al., 2018). Mean colostrum volume for Holstein and Jersey cows was within range of previous reports, 4.26 kg-26.5kg (Gavin et al., 2018). Holstein cows had average test-day SCS, protein, and milk within normal range (Negussie et al., 2013). Average test-day SCS, protein, fat, milk, colostrum lactoferrin, protein, fat, and lactose were within normal range for Jersey cows (Gavin et al., 2018; Tsuji et al., 1990; Morril et al; 2012). Solids non-fat and SCS for this study were 19% and 44% less than reported literature values (Yaylak et al., 2017). Additionally, mean colostrum urea concentration exceeded the recommended range of 10-14 mg/dL (Ishler, 2016). Even though colostrum Brix scores, volume, SCS, and solids non-fat differed from previous

literature, this data set can still be considered as a representative sample of the population because of their small standard errors indicating less spread in the data set.

Calf phenotypic data for this study showed that average birth weight for Holstein calves was 39.45. kg at birth while Jerseys calf average birth weight was 27.70 kg. Overall, average Brix score for first and second feeding was 24.87% and 23.01%, respectively, which was greater than the national guideline of 22% (Merial, 2018). Also, average amount of colostrum fed at first and second feeding was 3.17 L and 2.11 L, respectively. While initial feeding of colostrum was below the recommended 3.78 L, the average total amount fed (i.e feeding 1+2) was 5.25 L which was above the 3.78 L guideline (**Table 3.2**). Also, TSP for calves fed frozen colostrum was 6.32 g/dL while those fed fresh colostrum averaged 6.46 g/dL. Calves fed their dam's colostrum had TSP of 6.05 g/dL, those fed a mixture of dam and another cow's colostrum was 6.60 g/dL, and those fed other source of colostrum had 6.53 g/dL. Total serum protein levels in calves are, ideally, to be greater than 5.5 g/dL for successful passive immunity transfer (Morril et al., 2012). The overall average TSP for this study was 6.90 g/dL (HO calves: 7.03 g/dL; JE calves: 6.67 g/dL) with 92% of calves achieving passive immunity transfer.

Phenotypic analysis. Brix score and colostrum weight over time by breed are depicted in **Figures 3.1** and **3.2**. The main effects of breed ($P < 0.001$) and month ($P < 0.001$) differed by Brix score, but breed × month did not differ ($P = 0.095$). Mean colostrum Brix score for Holsteins in October was 27.26% which was significantly greater than the month of May and June with a 16% decrease in Brix score from October to the other months. For Jersey cows the month of October had a mean Brix score of 26.28% which was significantly greater than the months of May, June, July, and August with greater than a 22% decrease in Brix score when comparing June to the other months. For colostrum volume, the main effects of breed ($P <$

0.001) and month ($P < 0.001$) differed, and breed × month was significant ($P = 0.002$). Colostrum volume for Holstein and Jersey cows were significantly different for January ($P = 0.001$), February ($P = 0.005$), September ($P = 0.014$), and November ($P = 0.042$) with Holstein cows having greater colostrum volume produced for each month observed to be significantly different. Holstein mean colostrum volume was 12.56 kg, 8.93 kg, 8.42 kg, and 6.05 kg for the months of January, February, September, and November. Jersey cow mean colostrum volume was 2.46 kg, 4.19 kg, 4.19, and 2.35 kg for January, February, September, and November. Holstein cows produced mean colostrum volume of 4.82 kg in the month of December which was significantly less than the months of January and May by greater than 59%. Jersey cow colostrum volume had increased month to month variability with June having mean volume of 8.72 kg which is significantly greater than the months of January, February, December, August, September, and November. The difference between June colostrum volume and the other months was greater than 36%. Additionally, the month of May had mean colostrum volume of 7.4 kg which was significantly greater than the months of January, February, December, November, and September. The difference between May colostrum volume and other months was greater than 56%.

Results for Holstein and Jersey cows showed that colostrum volume ($P < 0.001$), lactose ($P < 0.001$), and lactoferrin ($P = 0.002$) varied significantly by season. Lactose content (3.32%) was highest during the winter, whereas lactoferrin (2.03 mg/mL) was lowest during the winter. Also, test day milk ($P = 0.046$), fat ($P = 0.012$), and protein ($P = 0.003$) varied significantly by season as well with all three components being in the greatest concentration during the winter months. Additionally, both colostrum Brix and volume ($P < 0.001$) differed by breed with Holstein cows having a greater Brix score by 12%. Holstein cows also had a greater colostrum

volume by 48%. Test-day components all differed by breed with the greatest difference being observed in milk yield. Moreover, a significant season and breed interaction ($P = 0.028$) was observed solely for colostrum volume.

Results for Pearson correlation showed significant ($P < 0.001$) strong correlations between true protein and solid non-fats ($r_p = 0.99$) and Brix ($r_p = 0.86$); solid non-fats also had a strong correlation with Brix (r = 0.85). Additionally, significant ($P < 0.001$) moderate correlations were observed between lactose and lactoferrin (r = -0.33), colostrum volume (r = 0.32), Brix (r = -0.48), true protein (r = -0.59), and solid non-fats (r = 0.47). These relationships support the use of Brix score as an indirect measurement of colostrum quality which is indicative of protein concentration in colostrum. Volume and Brix were also observed to have an inverse moderate correlation ($r_p = -19$) and we see the same relationship between Lactoferrin and volume and lactose which could point to lactoferrin being another measurement of quality (**Table 3.4**).

Calf phenotypic analysis showed incidence of scours was not impacted by season, TSP levels, freshness of colostrum fed, volume fed, brix of colostrum fed, colostrum source or colostrum components ($P > 0.05$). Furthermore, variables such as colostrum true protein ($P =$ 0004), SNF ($P < 0.001$), and TSP ($P = 0.038$) impacted respiratory illness, but management practices did not. Season was also marginally significant for respiratory illness ($P = 0.060$; **Table 3.5**).

Genetic Parameters. The estimates of variance components and heritability for Holstein and Jersey cow Brix score, colostrum weight, and test-day composition traits obtained through pedigree-based approaches are given in **Table 3.6** and **3.7**, respectively, whereas genetic and phenotypic correlations are shown in **Tables 3.8** and **3.9**. In Holstein cows, colostrum Brix score ($h^2 = 0.25$), fat ($h^2 = 0.27$), protein ($h^2 = 0.38$), and SCS ($h^2 = 0.24$) were moderately heritable,

whereas heritability estimates for colostrum volume (h^2 = 0.15) and test-day milk (h^2 = 0.19) were low. Heritability estimates in Jersey cows were moderate for all colostrum and test-day milk traits (h^2 = 0.33 to 0.55). Repeatability for quality traits and test-day component traits in Holstein and Jersey cows were moderate to high (r = 0.36 to 0.71; SD = 0.04 to 0.18).

Genetic correlations for Holstein cows were moderately correlated between colostrum Brix score and test-day milk, fat, and SCS (r_g = -0.23, 0.54, and 0.29); whereas, genetic correlations of colostrum Brix score compared to colostrum volume and protein were weak. Phenotypic correlations for Holstein cows showed moderate correlations between Brix score with volume and test-day milk, fat, and protein (r_p = -0.27, 0.16, 0.19, and 0.22), but weak correlations with SCS (r_p = -0.05). Genetic correlations between colostrum volume and test-day milk was strong (r_g = 0.96), whereas colostrum volume and test-day fat, protein, and SCS were moderately correlated (r_g = 0.46, 0.46, and -0.73). Colostrum weight for Holsteins showed moderate phenotypic correlations with SCS (r_p= -0.14), but weak correlation with test-day milk, fat, and protein (r_p = 0.02, 0.02, and 0.01). For Jersey cows, colostrum Brix score with colostrum volume and test-day fat and protein were strongly genetically correlated (r_g = -0.99, 0.91, 0.90), and moderate correlations were observed between colostrum Brix score and test-day milk and SCS (r_g = 0.42 and -0.20). Phenotypic correlations for Jersey cow Brix score were moderate with colostrum volume and protein (r_p = -0.25 and 0.13), and weak correlations with test-day milk, fat, and SCS (r_p = 0.09, 0.10, -0.03). Colostrum volume for Jersey cows was weakly correlated with all compositional traits except for SCS and fat, which was moderately correlated (r_g = -0.33 and -0.15). Phenotypic correlations in Jersey cows for colostrum volume with test-day components were all weak (r_p = -0.09 to 0.07).

DISCUSSION

For this study, the objectives were to 1) establish phenotypic relationships between colostrum composition traits, milk production traits, and calf health, 2) determine impact of breed and season on colostrum production and 3) elucidate the genetic parameters (i.e. heritability, genotypic, and phenotypic correlations) among colostrum production and milk production. Currently, there is a gap in the literature with regards to the relationships of colostrum components with calf health and long-term performance. Studies have focused on genetic parameters for milk fatty acid, milk yield, quality, body condition score, and production traits (Petrini et al., 2016; Loker et al., 2009; Kadarmideen and Wegmann, 2003; Ulutas et al., 2008). However, no known study has been conducted to determine the genetic parameters surrounding colostrum Brix score and colostrum volume for both the Holstein and Jersey breeds. Determining the genetic parameters of Brix score and colostrum volume would allow for selection to improve production of optimal colostrum quality and quantity thus promoting better calf health.

Descriptive Statistics. Discrepancies were observed for mean colostrum volume for both Holstein and Jersey cows compared to literature (Soufleri et al, 2019; Gavin et al., 2018). Additionally, discrepancies in Jersey cow mean colostrum Brix score were also observed. Differences in colostrum production can be attributed to management and environmental differences for each farm. Some of these differences can include diet and dry-period length (Nowak et al., 2012; Godden, 2019; Shoshani et al., 2014).

In our study, overall Brix score average for Jersey cows was below that reported by a nationwide study, 23.5% (Urie et al., 2019). Average Brix scores were 22.42, 24.55, and 18.63 for farm 1, 2, and 3. Brix score can be affected by factors like dam diet and dry-period length. Shoshani et al. (2014) reported significant decrease in colostrum protein, which is strongly

correlated with Brix score, in cows that had dry-periods shorter than 60 days. Farm 3 had a very low average Brix score which can be attributed to over 30% of the animals having less than 60 d dry periods. Colostrum production occurs several weeks pre-partum and is marked by the influx of immunoglobulins into the mammary gland. While little is understood about the physiological mechanisms surrounding colostrum production, impacts to influx of immunoglobulins has been observed when shortening the dry period (Mayasari et al., 2015). This could be due to loss of colostrum immune components due to continuous milking during the critical influx period during colostrogenesis. Additionally, Nowak et al. (2012) reported the importance of formulating differentiated diets for far off and close up dry cows to ensure optimal colostrum is produced. However, farm 3 reported that cows were fed the same diet for both far off and close up dry cows which can be contributing to such a low average Brix. Colostrum component synthesis is dependent on nutrition during the dry-period (Castro et al., 2011). Therefore, if the appropriate diet is not fed during the dry period biosynthesis of components like protein would be impacted and could lower Brix score. With farm 3 having such a low average Brix score this can be skewing the overall Jersey Brix average making it below nationwide average.

Colostrum volume national averages have yet to be determined. However, there have been numerous large studies that have reported mean colostrum yield ranges for multiple dairy breeds. Reported values ranged from 1.4 kg to 27.7kg (Parrish et al., 1950; Silva del Rio et al., 2017; Gavin et al., 2017). Additionally, Pritchett et al. (1991) reported that the optimal level of colostrum volume was 8.5 kg to ensure production of superior colostrum quality. While this study was conducted in Holstein and values might be breed specific there is no threshold determined for Jerseys. Therefore, Holstein cow threshold value can be used as a guideline. Our mean Holstein cow values fall within normal range of previously determined colostrum yield

values. However, for Jersey cows mean colostrum volume was below optimal level (8.5 kg) of colostrum volume at 6.03 kg. This held true for the winter (2.31 kg), summer (6.79 kg), and fall (4.04 kg) seasons. The spring was the only season where mean colostrum volume was above optimal colostrum volume at 9.54 kg. These changes in mean colostrum value by season could be attributed to differences to photoperiod (i.e. changes to daylight hours). Old (2014) reported that changes to daylight hours throughout the year can cause changes to body composition such as gaining weight during the fall and becoming leaner during the spring. Increases in mean volume observed for Jersey cows in the spring could be attributed to loss of body fat that is then used by the udder to increase volume (Old, 2014).

Environmental influences on colostrum quality traits. We observed breed differences in colostrum Brix score ($P < 0.001$) and volume ($P < 0.001$). This was expected as previous research observed differences in mean concentration of total immunoglobulin (%) among dairy cow breeds; Ayrshire averaged 8.1%, 6.6% for Brown Swiss, 6.3% for Guernsey, 5.6% for Holstein, and 9% for Jerseys (Guy et al., 1994). Based on these differences in total immunoglobulin by breed, variation in Brix score among breeds would be expected Brix score is an indirect measure of immunoglobulin concentration. Additionally, variation in the density of immunoglobulin by breed would also influence colostrum volume to different degrees. Breeds with increased concentrations of immunoglobulins would produce less colostrum volume. This same trend was observed by Kruse (1958) with Red Danish breeds IgG content compromising 5.6% of their colostrum when compared to Black and white Danish breeds (7.2%). However, Red Danish had the greatest colostrum yield (7.5 kg) when compared to Black and white Danish (5.3 kg). These breed differences could be attributed to potential differences in protein biosynthesis during colostrogenesis. Guy et al. (1994) reported something similar with potential

breed differences being attributed to differences in lactogenic activity of alpha-lactalbumin. Alpha lactalbumin is a component of lactose synthase, therefore, more alpha lactalbumin increases lactose content of colostrum which, in turn, increases water content and increases colostrum volume. Which leads to the observation that greater colostrum volume can result in a dilution effect thus decreasing IgG in colostrum. Holstein cows in our study produced 24% more colostrum than Jersey cows which could point to Holsteins having grater lactogenic activity which is why Holsteins produce the lowest IgG content of dairy breeds and Jerseys produce the greatest (Guy et al., 1994). Also, a significant interaction between breed and season was observed for colostrum volume pointing to volume being more heavily influenced by interaction of breed and season than either of these two factors separately.

Our research indicated that there were significant differences in Brix score by season for both breeds. In Holstein cows, fall season had a significantly greater ($P = 0.012$; 25.91%) Brix score average when compared to spring (23.73%), but there were no significant differences between fall, summer, and winter. Jersey cow mean colostrum Brix was significantly greater in the fall ($P = 0.008$; 23.69%) when compared to the summer (21.16%), but there were no significant differences with the spring (22.74%) and winter (22.33%). In literature seasonal changes in colostrum quality have been reported with fall having the greatest immunoglobulin content when compared to other seasons as observed for both breeds cows from our study (Gulliksen et al., 2008). Colostrum volume was impacted by season for Jersey cows but not Holstein cows. Jersey cow mean colostrum volume in the spring (8.5 kg) was significantly greater than fall ($P = 0.001$; 5.1 kg) and winter ($P < 0.001$; 4.1 kg) but was not significantly different than summer ($P = 0.077$; 6.7 kg). Spring months had the greatest mean colostrum volume with greater than a 23% difference when compared to summer, fall, and winter.

Additionally, increased volume of colostrum ($P = 0.043$) was produced in summer months compared to winter months with a 38% decrease from summer to winter. Zarei et al. (2017) reported that Holstein had greater colostrum volume in the spring (7.7 kg) and the lowest volume in the fall (6.5 kg). These findings differ from our Holstein cow results; however, they were similar to our results for colostrum volume in Jersey cows with the greatest volume being produced in the spring. Additionally, Gavin et al. (2018) reported that colostrum volume in Jersey cows gradually decreased from summer to winter months (6.6 kg to 1.3 kg). It follows the trend observed in our Jersey cows where colostrum volume was greatest in the spring but lowest in the winter. Differences in farm management practices such a diet fed and dry cow management might be responsible for why these discrepancies were observed (Godden et al, 2019). Seasonal variation of cow diet and feed regimes due to feed availability and quality throughout a given year could impact the quality and quantity of colostrum produced by disrupting normal biosynthetic pathways of colostrum components (Nateghi et al., 2014). Also, Shoshani et al. (2014) observed a significant decrease in milk yield, fat, and protein in cows with dry periods less than 60 d. In our study some cows had dry periods of less than 40 days long that could impact the transport of components into the mammary gland during the dry period and decrease yield of colostrum depending on their calving season.

Environmental impacts on Colostrum composition. Colostrum quality traits can also be impacted by variation in colostrum composition, therefore, components such as colostrum protein, fat, SCS, lactose, lactoferrin, and urea were quantified for Jersey cows. Colostrum lactose ($P < 0.001$) and lactoferrin ($P = 0.002$) were the only components found to be significantly impacted by season. Lactose is the major osmole in milk, therefore, with seasonal variation in lactose a change in colostrum volume is expected just as we observed for our Jersey

cows. Lactose and colostrum volume were observed to have a moderate positive correlation (r_p = 0.32; $P < 0.001$) and we can also observe this relationship when comparing lactose content by season with spring having the greatest lactose content (3.16%) and also the greatest mean colostrum volume. Moreover, lactoferrin is an antibacterial, antimicrobial and antifungal protein in colostrum that bolster innate immunity and assist in the development of healthy gut microbiota (Sovereign Laboratories, 2017). This is indicative of a crucial component within colostrum that contributes to colostrum quality. Lactoferrin was observed to have moderate correlations with both Brix score and colostrum volume. The correlation between Brix and lactoferrin was $r_p = 0.20$ indicating that as Brix score increases so does lactoferrin. Additionally, the correlation between lactoferrin and colostrum volume was $r_p = -0.35$ indicating an inverse relationship with a decrease in colostrum being associated with an increase in lactoferrin.

These relationships were observed by season as well. Fall had the greatest mean Brix score (24.52%) and greatest colostrum lactoferrin content (5.03 mg/mL), but one of the lowest mean colostrum volumes (6.35 kg) in our analysis. Summer was the season with the second greatest lactoferrin content (4.15 mg/mL) with a Brix of 23.18% and volume of 7.68 kg. These relationships are expected since Brix score is an indirect measure of protein content and lactoferrin is an important protein in colostrum, therefore, an impact to Brix score would make sense. Furthermore, the decrease in colostrum volume associated with changes in lactoferrin concentration was also expected because of the significant inverse relationship observed in phenotypic measurements of this study. Additionally, phenotypic analysis was conducted to assess relationships between environment and colostrum composition on incidence of scours and respiratory illness in calves. Overall, 93% of calves had successful passive immunity transfer (TSP > 5.5 g/dL) and mortality rate was 11% for this study. The calves were fed above

recommended quantity of colostrum at 5.2 L and above national guideline quality of colostrum at 24.87 Brix percentage.

Incidence of scours was not significantly impacted by any other factor in our model. However, factors such as TSP ($P = 0.038$), colostrum true protein ($P < 0.001$), and SNF ($P < 0.001$) were all associated with incidence of respiratory illness which is supported by literature (Aydogdu and Guzelbektes, 2018; Meganck et al., 2014). Higher protein content in colostrum (i.e. true protein and SNF) is associated with less incidence of disease and greater success of passive immunity (Aydogdu and Guzelbektes, 2018; Meganck et al., 2014). Moreover, studies have shown that TSP can act as an accurate predictor for calf incidence of disease and mortality within the first 14 wks of life (Donovan et al., 1986). Therefore, the observation that TSP significantly impacted incidence of respiratory illness was expected. While our model did not indicate colostrum freshness as a significant factor Stieler et al. (2012) reported calves fed frozen colostrum had high production of neutrophil and immune cells and less incidence of disease when compared to calves given fresh colostrum. Also, lactoferrin was not significant in the model, however, Habing et al. (2017) reported that lactoferrin administered to calves after becoming ill significantly decreased mortality and culling. This presents an innovative way to improve treatment plans that exist for both scours and respiratory illness. Perhaps lactoferrin does not play a role in prevention but can serve as a tool to treat illness still indicating that lactoferrin is an important component in colostrum. Collectively, these results provide further insight for use of colostrum components in combination with other established farm practices as another target for modulation of colostrum quality and quantity.

Genetic parameters. Elucidating genetic relationships of colostrum production is of importance to the dairy industry. Being able to establish these relationships would 1) help

determine the degree of environmental influences on traits versus gene regulation and 2) allow us to determine which traits are inherited together or impact each other. Additionally, determining what traits have the greatest impact on long-term animal performance would help in selection of traits favoring producer's production goals and improvement of desired traits which can lead to both genetic and economic gain. Heritability estimates for Brix score ($h^2 = 0.27 \pm 0.09$) and colostrum volume ($h^2 = 0.04 \pm 0.06$ to 0.37) have been previously calculated (Ulutas et al., 2008; Soufleri et al., 2019), which reflect findings in our study ($h^2 = 0.15$ to 0.47) for both breeds. Additionally, repeatability estimates, the extent to which variation in an individual contributes total variation in a population, were measured in this study (Boake, 1989). Estimates are used to predict future performance from past animal records. In this data set repeatability estimates ranged from $r = 0.38$-0.71 which is considered moderate to high repeatability. Based on repeatability's observed in the data, selection for quality traits can be made based on the first record of the trait, and it would prove effective in improving over-all performance of the herd in the following year. The moderate heritability observed in this study also indicated that repeated measures for quality traits of a given animal have less variation than measure of other individual animals. High repeatability also indicates low environmental variation and that genetic variation is additive in nature (Boake, 1989). Test-day milk heritability in Holsteins was moderately low at 0.19. Petrini et al. (2016) reported this same trait to have moderately low heritability at 0.12. For Jerseys, test-day milk was found to be moderately heritable at 0.33, whereas in a study by Roveglia et al. (2018) the heritability was 0.14 and lowly heritable. For Holstein and Jersey cows, the remaining test-day traits such as fat, SCS, and protein have heritability values reported by Heinrichs (2016), Roveglia et al. (2016), and Petrini et al. (2016) and no discrepancies were observed. Most of the heritability's for traits in this study (i.e. Brix, volume, test-day

components) were moderate to high which indicate that most of the variation for each particular trait can be attributed to genetic differences of individual animals. Heritability values can be used in breeding programs by producers and allows them to determine which animals are expected to have superior phenotypic performance in order to improve a trait. Additionally, heritability can provide insight into how much variation of a specific trait is controlled by environment or management practices. Also, genetic merit for an animal can also be predicted based on heritability by measuring the expected change in breeding value (Cassel, 2009).

When formulating selection indices to ensure proper weighting of traits it is important to avoid unfavorable outcomes with other economically relevant traits. Consideration must be given to the overall production and management goals of the herd. This is because when genetic correlations are strong, selection for one trait impacts another trait due to overlaps in gene regulation. The greater the genetic correlation value the greater number of genes they share that affect both traits, whereas the closer to zero correlations are the less genes they share (Cassell, 2009). Additionally, moderate genetic correlations (± 0.2 to 0.6) are in middle ground while they may be used for selection the progress can be slow or make it difficult to improve traits that are difficult or expensive to measure (Cassel, 2009).

Genetic correlation for colostrum Brix score and colostrum volume with test-day compositional traits for our study ranged from weak to strong for Holstein cows. We observed a positive but weak genetic correlation between Brix score and colostrum volume (r_g = 0.06), but a negative but moderate phenotypic correlation (r_p = -0.27) for Holstein cows. This differed from Soufleri et al. (2019) who determined a positive but moderate genetic correlation (r_g = 0.49) and a negative weak phenotypic correlation (r_p = -0.10) between brix score and colostrum volume. Discrepancies can be explained by differences in sample size and familial relationships of cows

used in our study. Additionally, genetic correlation for Brix and volume is slightly greater than the phenotypic correlation which leads to the speculation that there might be imprecise estimation of phenotypic correlations due to small dataset.

The genetic correlation for Jersey cows between Brix score and colostrum volume was positive and strong (r_g = -0.99) and the phenotypic correlation was negative and moderate (r_p = -0.25). To date, there are no known studies in Jersey cows that have reported genetic or phenotypic correlations between Brix score and colostrum volume. Discrepancies between genetic and phenotypic correlation estimates may be due to missing records, pedigree, relationships both among and within farms, and a small sample size. Missing animal pedigree and records can impact correlation estimates by decreasing accuracy of estimations and causing models to either under or overestimate genetic variances which shows the importance of being able to link animal lineages. Finally, a difference in correlation estimates was observed between Holstein and Jersey cows while physiological processes are similar within species the process of colostrogenesis has not been heavily studied. Therefore, there could be underlying differences during colostrogenesis that could account for differences among breeds. Guy et al. (1994) reported that breed differences can potentially be attributed to differences in lactogenic activity of alpha-lactalbumin which can impact colostrum volume and subsequently colostrum Brix score by dilution effect of immunoglobulins. We have elucidated certain breed differences among Holstein and Jersey cows, however, alpha lactalbumin was not measured in our study. We were able to determine that genetic selection for colostrum quality traits was possible by calculating heritability, repeatability, genetic and phenotypic correlations. Heritability, genetic and phenotypic correlations between colostrum traits, and repeatability have been calculated which moves us one step closer to developing a selection index for colostrum quality traits. However,

more records on further generations are needed in order to determine estimated breeding values for individual animals.

CONCLUSION

We hypothesized that colostrum composition and production differ among breeds and by season and that individual components influence calf health. Additionally, we hypothesized that genetic improvement of colostrum quality is possible. Collectively, our results support our hypothesis as colostrum composition is associated with respiratory illness. Colostrum quality traits, which are important for calf health, were impacted by factors such as breed and season. Therefore, they should be considered in colostrum management practices. Genetic variance estimates and heritability were calculated for this study for colostrum quality traits as well as test-day component traits. Our study showed that both colostrum volume and Brix score were low to moderately heritable in both breeds making them receptive to genetic selection in order to improve breeding programs. We also observed weak genetic correlations between specific test-day component traits and quality traits which leads us to believe that when incorporated into a breeding program one may be able to select for traits such as colostrum weight and Brix score without having a drastic effect on component traits. However, due to the many discrepancies between studies, results with high standard errors, a small data set, and the lack of estimates for Jerseys we recognize the need for more in-depth studies before incorporating these traits into breeding programs. Our study also shed light on differences among breeds making it increasingly evident of an underlying mechanistic difference when it comes to colostrum production.

3. Figures and Tables

Table 3.1. Descriptive statistics for Holstein and Jersey colostrum quality and compositional traits

	Holstein (n = 250)			Jersey (n= 289)		
Variable	Mean ± SE[a]	Min	Max	Mean ± SE	Min	Max
Colostrum						
Brix score, %	24.43 ± 0.23	2.00	31	22.39 ± 0.28	10.00	31.00
Colostrum Volume, lb	17.52 ± 0.66	1.00	63	13.27 ± 0.49	1.00	53.00
Lactoferrin, mg/ml	.	.	.	2.38 ± 0.22	0.006	12.12
Protein, %	.	.	.	13.22 ±0.25	4.17	20.06
Fat, %	.	.	.	4.63 ± 0.14	0.61	14.60
Lactose, %	.	.	.	2.92 ± 0.04	1.20	4.46
SNF[b], %	.	.	.	17.01 ± 0.23	8.11	23.68
SCS[c], cells/ml	.	.	.	3.75 ± 2.63	1.51	4.48
Urea, mg/dL	.	.	.	34.35 ± 0.90	0.50	68.20
Test-day						
Test-day milk, lb	86.11 ± 1.52	8.90	161.00	64.04 ± 0.95	21.40	114.00
Test-day protein, %	2.79 ± 0.04	0.38	4.77	2.23 ± 0.03	0.84	3.99
Test-day fat, %	3.56 ± 0.06	0.33	6.57	2.84 ± 0.04	0.90	5.68
Test-day SCS, $\log_{10}$	2.50 ± 0.12	0.10	9.50	3.46 ± 0.11	0.10	9.50
Dam age, yrs	5.87 ± 0.11	3.00	15.00	6.86 ± 0.15	3.00	21.00

[a]Standard error
[b]Solid non-fats
[c]Somatic cell score

Table 3.2. Descriptive statistics on Holstein and Jersey calf records

	Holstein			Jersey		
Variable	Mean ± [a]SE	Min	Max	Mean ± SE	Min	Max
Calf Records						
Birth Weight, kg	39.45 ± 1.07	21.36	49.54	27.70 ± 1.03	19.09	46.36
[b]TSP, g/dL	7.03 ± 0.07	4.80	8.00	6.67 ± 0.13	3.40	8.80
First Feeding amount, Q	3.60 ± 0.07	1.00	5.00	3.00 ± 0.10	1.00	5.00
Total Colostrum Fed, Q	5.36 ± 0.10	2.00	8.00	5.38 ± 0.18	1.00	8.00

[a]Standard Error
[b]Total Serum Protein

Table 3.3 Quality traits, colostrum and test day components LSMEANS for Breed and Season

Item	Holstein	Jersey	Breed *P*-value	Fall	Spring	Summer	Winter	Season *P*-value	Season × Breed *P*-value
Quality traits									
Brix, %	24.92 ± 0.34	22.31 ± 0.36	< 0.001	24.52 ± 0.58^{a}	23.36 ± 0.36^{a}	23.18 ± 0.47^{a}	23.40 ± 0.52^{a}	0.188	< 0.061
Volume, kg	8.15 ± 0.68	5.50 ± 0.70	< 0.001	6.99 ± 1.14^{bc}	8.12 ± 0.70^{ab}	7.68 ± 0.92^{abc}	5.16 ± 1.02^{cd}	< 0.001	0.028
Colostrum									
Protein, %	.	.	.	14.20 ± 0.56^{a}	13.60 ± 0.54^{a}	13.81 ± 0.48^{a}	12.73 ± 0.47^{a}	0.299	.
Fat, %	.	.	.	4.12 ± 0.55^{a}	4.28 ± 0.54^{a}	4.12 ± 0.48^{a}	5.19 ± 0.47^{a}	0.550	.
SNF[3], %	.	.	.	17.58 ± 0.53^{a}	17.30 ± 0.51^{a}	17.42 ± 0.45^{a}	16.91 ± 0.45^{a}	0.827	.
Lactose, %	.	.	.	2.52 ± 0.13^{c}	3.16 ± 0.12^{ab}	2.74 ± 0.11^{ab}	3.12 ± 0.11^{d}	< 0.001	.
SCS[4], Log_{10}	.	.	.	3.86 ± 3.22^{a}	3.74 ± 3.20 ^{a}	3.76 ± 3.16^{a}	3.77 ± 3.15^{a}	0.687	.
Urea, mg/dL	.	.	.	36.38 ± 3.46^{a}	39.76 ± 3.34^{a}	38.05 ± 2.98^{a}	34.94 ± 2.94^{a}	0.678	.
Lactoferrin, mg/mL				5.03 ± 0.72^{bc}	2.80 ± 0.68^{ad}	4.15 ± 0.61^{bc}	2.03 ± 0.59^{ad}	0.002	
Test-day									
Milk Yield, kg	37.73 ± 1.52	28.81 ± 1.56	< 0.001	31.75 ± 2.51^{abc}	33.35 ± 1.57^{abcd}	32.14 ± 2.03^{abc}	35.84 ± 2.28^{ad}	0.046	0.184
Protein, %	2.65 ± 0.04	2.19 ± 0.04	< 0.001	2.30 ± 0.08^{abc}	2.47 ± 0.04^{abc}	2.29 ± 0.06^{abc}	2.62 ± 0.06^{ad}	0.003	0.395
Fat, %	3.42 ± 0.07	2.73 ± 0.07	< 0.001	2.96 ± 0.13^{abcd}	3.06 ± 0.07^{abc}	2.89 ± 0.09^{abc}	3.39 ± 0.11^{acd}	0.012	0.550
SCS, Log_{10}	2.76 ± 0.17	3.52 ± 0.16	0.001	353 ± 0.28^{a}	2.93 ± 0.16^{a}	3.30 ± 0.21^{a}	2.79 ± 0.23^{a}	0.156	0.624

[1]Estimates provided are LSMEANS ± SE ; model = Quality traits, colostrum and test-day components dependent variables with breed, season , and interaction as independent variables and parity, dam age, calving year as covariates

[2]Superscirpts a,b,c,d indicate significant differences among the seasons (Spring = a, Summer = b, Fall = c, Winter = d)

[3]Solid non-fats

[4]Somatic cell score

Table 3.4. Phenotypic correlation[1] among colostrum compositional traits with correlations (r_p) on top and corresponding *P*-value below.

Variables	Brix	Volume	Lactoferrin	Fat	TP[2]	SNF[3]	Lactose	SCS[4]	Urea
Brix	-	-0.19[5]	0.20	0.08	0.86	0.85	-0.48	-0.26	0.40
		0.002[6]	0.027	0.203	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Volume	-0.19	-	-0.33	0.02	-0.22	-0.18	0.32	-0.17	-0.16
	0.002		< 0.001	0.694	< 0.001	0.004	< 0.001	0.009	0.012
Lactoferrin	0.20	-0.33	-	-0.11	0.24	0.20	-0.33	0.02	-0.12
	0.027	< 0.001		0.232	0.008	0.026	< 0.001	0.795	0.170
Fat	0.08	0.02	-0.11	-	0.01	0.02	0.07	-0.03	0.19
	0.203	0.694	0.232		0.856	0.687	0.239	0.636	0.003
TP	0.86	-0.22	0.24	0.01	-	0.99	-0.59	-0.15	0.47
	< 0.001	< 0.001	0.008	0.856		< 0.001	< 0.001	0.019	< 0.001
SNF	0.85	-0.18	0.20	0.02	0.99	-	0.47	-0.22	0.49
	< 0.001	0.004	0.026	0.687	< 0.001		< 0.001	0.009	< 0.001
Lactose	-0.48	0.32	-0.33	0.07	-0.59	0.47	-	-0.28	-0.19
	< 0.001	< 0.001	< 0.001	0.239	< 0.001	< 0.001		< 0.001	0.003
SCS	-0.26	-0.17	0.02	-0.03	-0.15	-0.22	-0.28	-	-0.09
	< 0.001	0.009	0.795	0.636	0.019	0.009	< 0.001		0.149
Urea	0.40	-0.16	-0.12	0.19	0.47	0.49	-0.19	-0.09	-
	< 0.001	0.012	0.170	0.003	< 0.001	< 0.001	0.003	0.149	

[1]Pearson correlation (r_p)
[2]True Protein
[3]Solids non-fat
[4]Somatic cell score
[5]Value depicts correlation coefficient
[6]$P < 0.05$

Table 3.5. Impact of colostrum components and season with health measures in Jersey calves

	Scours		Respiratory Disease	
Variable	LSMEAN ± SE[a]	*P* - value	LSMEAN ± SE	*P* – value
Environment				
Season	-0.42 ± 0.28	0.134	-0.53 ± 0.28	0.060
Total Serum Protein, mg/dL	0.10 ± 0.13	0.420	-0.27 ± 0.133	0.038
Management				
Feeding Freshness (Fresh vs. Frozen)	0.08 ± 0.08	0.301	0.05 ± 0.08	0.487
Feeding Source (dam vs mix)	-0.14 ± 0.25	0.577	0.03 ± 0.25	0.895
Brix of Colostrum fed, %	-0.33 ± 0.52	0.520	-0.56 ± 0.52	0.286
Volume Fed, L	0.10 ± 0.14	0.453	0.15 ± 0.14	0.285
Colostrum Component				
Protein, %	-0.93 ± 1.04	0.377	-3.44 ± 0.85	< 0.001
Fat, %	0.25 ± 0.85	0.766	0.15 ± 0.85	0.861
SNF[b], %	-0.99 ± 0.98	0.332	-3.39 ± 0.80	< 0.001
Lactose, %	-0.03 ± 0.19	0.858	0.04 ± 0.19	0.824
SCS[c], Log_{10}	2.30 ± 3.27	0.915	3.45 ± 3.26	0.128
Urea, mg/dL	-3.52 ± 4.13	0.398	0.08 ± 4.20	0.996
Lactoferrin, mg/mL	0.2844 ± 1.90	0.883	-0.57 ± 1.88	0.766

[a]Standard error
[b]Solid non-fats
[c]Somatic cell score

Table 3.6. Additive genetic variance (σ^2_a), permanent environmental variance (σ^2_{pe}), error variance (σ^2_e), heritability (h^2), and repeatability (r) of Brix score, colostrum volume, and test-day milk, fat, protein, and SCS yields in Holsteins cows.[1]

	σ^2_a		σ^2_{pe}		σ^2_e		h^2		R	
	Estimate	SE	Estimate	SE	Estimate	SE	Mean	SD	Mean	SD
Brix	5.40	3.12	8.71	2.72	1.91	0.51	0.33	0.18	0.88	0.04
Volume	16.90	19.43	60.17	19.86	30.12	7.35	0.15	0.18	0.71	0.07
Milk	123.39	127.93	179.67	137.51	324.90	82.39	0.19	0.20	0.48	0.14
Fat	0.31	0.24	0.43	0.22	0.39	0.10	0.27	0.20	0.65	0.10
Protein	0.18	0.12	0.08	0.11	0.19	0.04	0.39	0.24	0.57	0.13
SCS	1.01	0.80	0.60	0.91	2.48	0.68	0.24	0.19	0.39	0.18

[1]Sampling means and variances for h^2 and r are based on 10,000 replicates.

Table 3.7. Additive genetic variance (σ^2_a), permanent environmental variance (σ^2_{pe}), error variance (σ^2_e), heritability (h^2), and repeatability (r) of Brix score, colostrum volume, and test-day milk, fat, protein, and SCS yields in Jersey cows.[1]

	σ^2_a		σ^2_{pe}		σ^2_e		h^2		r	
	Estimate	SE	Estimate	SE	Estimate	SE	Mean	SD	Mean	SD
Brix	9.23	3.31	0.001	0.02	15.86	3.71	0.37	0.14	0.37	0.14
Volume	31.78	12.88	4.02	14.57	30.69	10.33	0.48	0.18	0.53	0.16
Milk	76.34	39.89	25.88	49.43	121.53	38.08	0.34	0.17	0.45	0.17
Fat	0.24	0.12	0.14	0.13	0.31	0.09	0.35	0.17	0.54	0.14
Protein	0.11	0.05	0.02	0.06	0.14	0.05	0.39	0.38	0.48	0.17
SCS	0.23	0.50	2.60	0.74	1.33	0.91	0.06	0.06	0.68	0.13

[1]Sampling means and variances for h^2 and r are based on 10,000 replicates.

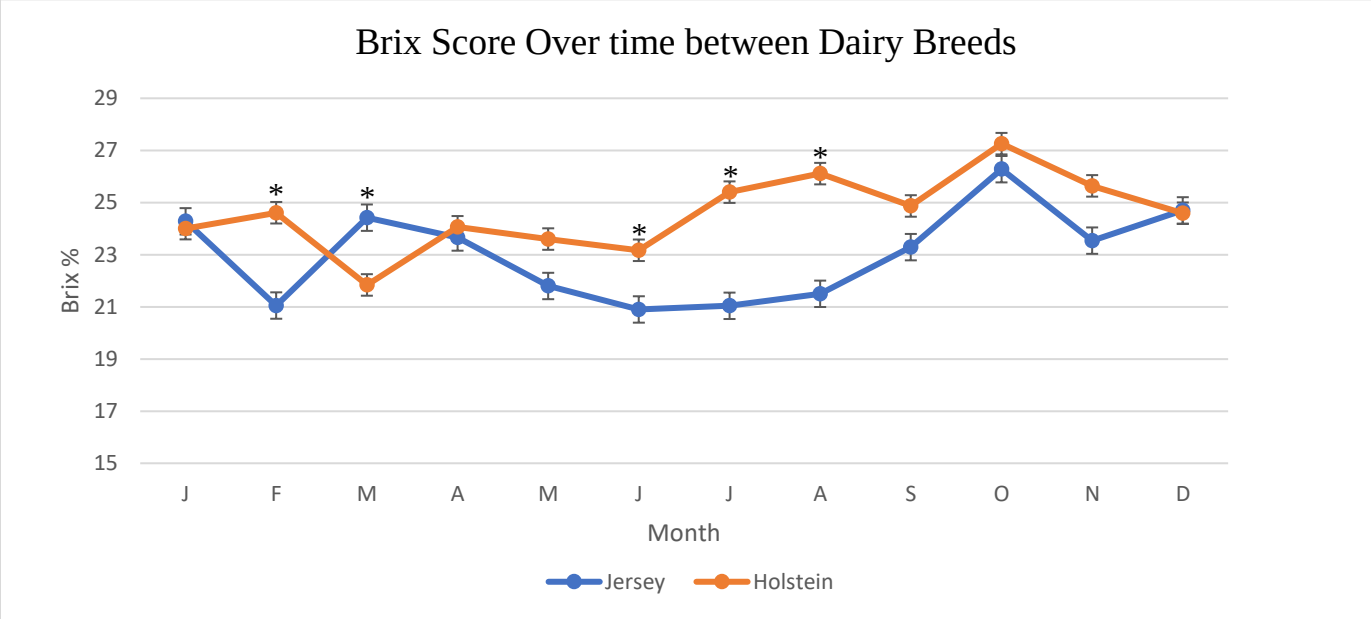
Table 3.8. Genetic (above the diagonal) and Phenotypic (below the diagonal) correlations of Brix score and colostrum volume with test-day milk traits for Holstein cows.

	Brix (%)	Volume (Kg)	Milk (Kg)	Fat (%)	Protein (%)	SCS (Log_{10})
Brix		0.06	-0.24	0.54	0.05	0.30
Volume	-0.27		0.97	0.46	0.47	-0.74
Milk	0.16	0.02		–	–	–
Fat	0.19	0.02	–		–	–
Protein	0.22	0.01	–	–		–
SCS	-0.05	-0.14	–	–	–	

Table 3.9. Genetic (above the diagonal) and phenotypic (below the diagonal) correlations of Brix score and colostrum volume with test-day milk traits for Jersey cows.

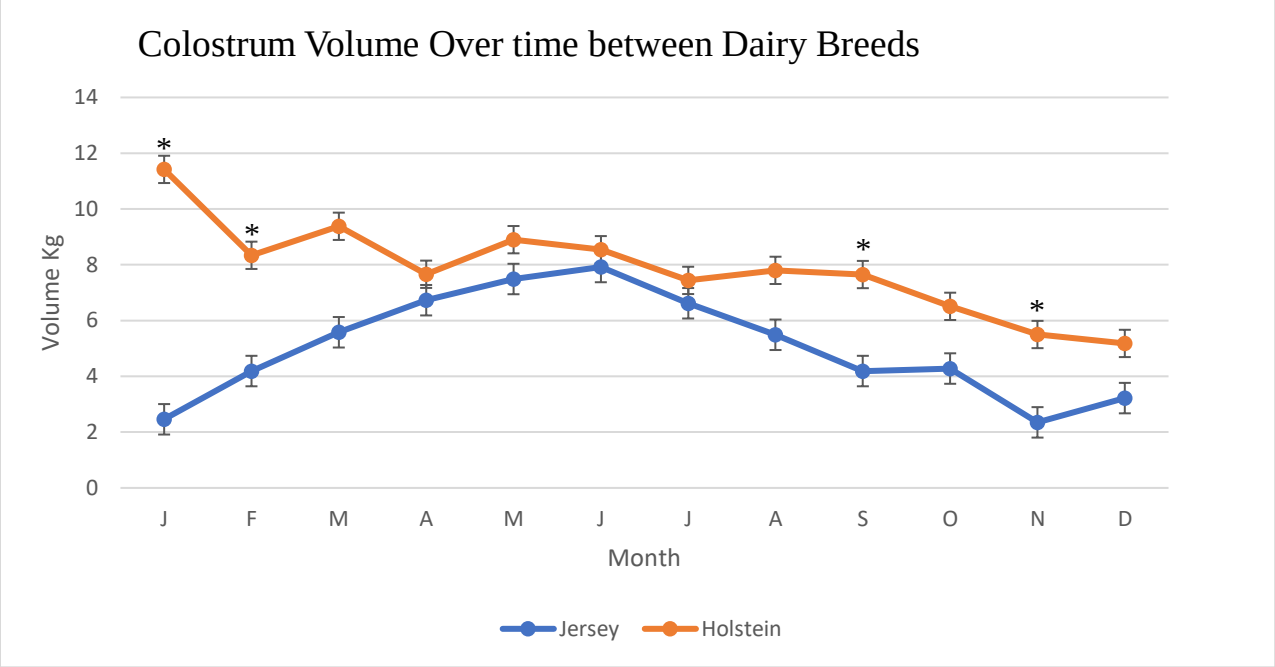
	Brix (%)	Volume (Kg)	Milk (Kg)	Fat (%)	Protein (%)	SCS (Log_{10})
Brix		-0.99	0.43	0.91	0.90	-0.21
Volume	-0.25		0.01	-0.15	0.04	-0.33
Milk	0.09	0.01		–	–	–
Fat	0.10	0.07	–		–	–
Protein	0.13	0.01	–	–		–
SCS	-0.03	-0.09	–	–	–	

Figure 3.1



*Significant difference in Brix score between breeds within month

Figure 3.2.



*Significant difference in Brix score between breeds within month

REFERENCES

Aydogdu, U., & Guzelbektes, H. (2018). Effect of colostrum composition on passive calf immunity in primiparous and multiparous dairy cows. *Veterinari Medicina*. 63(1), 1-11. doi: 10.17221/40/2017-VETMED

Boake, C. R. B. (1989). Repeatability: its role in evolutionary studies of mating behavior. *Evolutionary Ecology, 3*, 173-182.

Boland, M. (2011). Handbook of Food Proteins: Whey proteins. Food Science, Technology, and Nutrition. 30-55, doi:10.1533/9780857093639.30

Cassell, B. (2009). Using heritability for genetic improvement. *Virginia Cooperative Extension*.

Castro, N., Capote, J., Bruckmaier, R.M., & Argüello, A. (2011). Management effects on colostrogenesis in small ruminants: a review, *Journal of Applied Animal Research*, 39:2, 85-93, doi: 10.1080/09712119.2011.581625

Dahl, W.J. (2012). Gut microbiota and milk products: implications for health.

Donovan, G. A., Badinga, L., Collier, R. J., Wilcox, C. J., & Braun, R. K. (1986). Factors influencing passive transfer in dairy calves. *Journal of dairy science*, *69*(3), 754–759. https://doi.org/10.3168/jds.S0022-0302(86)80464-7

Gavin, K., Neibergers, H., Hoffman, A., Kiser, J. N., Cornmesser, M. A., Haredasht, S. A., . . . Moore, D. A. (2017). Low colostrum yield in Jersey cattle and potential risk. *J. Dairy Sci, 101*(7), 6388-6398. doi:10.3168/jds.2017-14308

Godden, S. (2019). Colostrum managment for dairy calves. *Vet Clin Food Anim, 24*, 19-39. doi:10.1016/j.cvfa.2007.10.005

Gulliksen, S.M., Lie, K.I., Solverod, L., & Osteras, O. (2008). Risk factors associated with colostrum quality in Norwegian Dairy cows. *J. Dairy Sci*. 91(2), 704-712. doi: 10.3168/jds.2007-0450

Guy, M.A., McFadden, T.B., Cockrell, D.C., & Besser, T.E. (1994). Regulation of colostrum formation in beef and dairy cows. *J. Dairy Sci*. 77, 3002-3007

Habing, G., Harris, K., Shuenemann, G.M., Pineiro, J.M., Lakritz, J., & Alcaraz Clavijo, X. (2017). Lactoferrin reduces mortality in preweaned calves with diarrhea. J. Dairy Sci. 100(5), 3940-3948. doi:10.3168/jds.2016-11969

Heinrichs, J., & Jones, C. M. (2016, 05/05/2016). Milk Components: Understanding milk fat and protein variation in your dairy herd. *PennState Extension*.

Ishler, V.A. (2016, 02/16/2016). Interpretation of milk urea nitrogen values. *PennSate Extension*.

Kadarmideen, H. N., & Wegmann, S. (2003). Genetic parameters for body condition score and its relationship with type and production traits in swiss holsteins. *86*, *86*(11), 3685-3693. doi:10.3168/jds.S0022-0302(03)73974-5

Kehoe, S.I. (2006). Colostrum components and their impact on digestive function and growth of dairy calves. The Pennsylvania State University Graduate school [Thesis 5/6/2020]

Kruse, V. (1958). Yield of colostrum and immunoglobulin in cattle at the first milking after parturition. Anim. Prod. 12, 619-626.

Sovereign Laboratories. (2017). Benefits of Lactoferrin in Bovine Colostrum. Retrieved from https://www.sovereignlaboratories.com/blog/benefits-lactoferrin-bovine-colostrum/

Loker, S. F., Bastin, C., Miglior, F., Sewalem, A., Fatehi, J., & Jamrozik, L. R. (2009). Genetic parameters of body condition score and milk production traits in Canadian Holsteins: . *Dairy Cattle Breeding and Genetics Meeting, Centre for Genetic Improvment of Livestock, University of Guelph (Canada)*, 11.

Mayasari, N., de Vreis Reilingh, G., Nieuwland, M.G.B., Remmelink, G.J., Parmentier, H.K., Kemp, B., & van Knegsel, A.T.M. (2015). Effect of maternal dry period length on

colostrum immunoglobulin content and on natural and specific antibody titers in calves. J. Dairy. Sci. 98(6), 3969-3979. doi: 10.3168/jds.2014-8753

Meganck, V., Hoflak, G., & Opsomer, G. (2014). Advances in prevention and therapy of neonatal dairy calf diarrhea: a systematical review with emphasis on colostrum management and fluid therapy. Acta Vet Scand. 56(1), 75. doi: 10.1186/s13028-014-0075-x

Morril, K. M., Conrad, E., Lago, A., Campbell, J., Quigley, J., & Tyler, H. (2011). Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *J. Dairy Sci., 95*, 3997-4005. doi:10.3168/jds.2011-5174 Nateghi, L., Yousefi, M., Zamani, E., Gholamina, M., & Mohammadzadeh, M. (2014). The effect of different season on the milk quality. *Euro. J. Bio*. 4(1), 550-552.

Negussie, E., Stranden, I., & Mantysaari, E. A. (2013). Genetic associations of test-day fat: Protein ratio with milk yield, fertility, and udder health traits in Nordic Red cattle. *J. Dairy Sci, 96*(2), 1237-1250. doi: 10.3168/jds.2012-5720

Nowak, W., Mikula, R., Zachwieja, A., Paczynska, K., Pecka, E., Drzaga, K., & Slosarz, P. (2012). The impact of cow nutrition in the dry period on colostrum quality and immune status of calves. *Polish Journal of Veterinary Sciences, 15*(1), 77-82. doi:10.2478/v10181-011-0117-5

Old, C. (2014). Seasonal changes in milk production. *Progressive Dairy*

Parrish, D.B., Wise, G.H., Hughes, J.S., & Atkeson, F.W. (1950). Properties of the colostrum of the dairy cow. Yield, specific gravity, and concentrations of total solids and its various components of colostrum and early milk. *J. Dairy Sci*. 33(6), 457-465. doi: 10.3168/jds.S0022-0302(50)91921-7

Petrini, J., Lung, L. H. S., Rodriguez, M. A. P., Salvian, M., Pertille, F., Rovadoscki, G. A., . . . Mourao, G. B. (2016). Genetic parameters for milk fatty acids, milk yield, and quality traits of Holstein cattle population reared under tropical conditions. *J. Anim Breed. Genet.*, 1-12. doi:10.1111/jbg.12205

Pritchett, L.C., Gay, C.C., Besser, T.E., & Hancock, D.D. (1991). Management and production factors influencing immunoglobulin G concentration in colostrum from Holstein cows. J. Dairy Sci. 74, 2336-2341.doi: 10.3168/jds.S0022-0302(91)78406-3

Robblee, E.D., Erickson, P.S., Whitehouse, N.L., McLaughlin, A.M., Schwab, C.G., Rejman, J.J., & Rompala, R.E. (2003). Supplmental Lactoferrin Improves Health and Growth of Holstein Calves during the Preweaning Phase. *J. Dairy Sci*. 86(4), 1458-1464. doi: 10.3168/jds.S0022-0302(03)73729-1

Robison, J. D., Stott, G. H., & Denise, K. (1988). Effects of passive immunity on growth and survival in the dairy heifer. *J Dairy Sci, 71*, 1283-1287.

Rocha, T. G., Franciosi, C., Nociti, R. P., Silva, P. C., Sampaio, A. A. M., & Fagliari, J. J. (2014). Influence of parity on concentrations of enzymes, proteins, and minerals in the milk of cows. *Arq. Bras. Med. Vet. Zootec, 66*(1), 315-320.

Roveglia, C., Costa, A., Penasa, M., Finocchiaro, R., Marusi, M., Lopez-Villalobos, N., & Cassandro, M. (2017). *Genetics of milk traits including lactose and somatic cell score in Italian Jersey cows.* Paper presented at the Proceedings of the World Congress on Genetics Applied to Livestock Production.

Shiksha, K. (2011). Principles of animal genetics and population genetics. *University of Georgia*

Shoshani, E., Rozen, S., & Doekes, J. J. (2014). Effect of a short dry period on milk yield and content, colostrum quality, fertility, and metabolic status of Holstein cows. *Journal of dairy science*, *97*(5), 2909–2922. https://doi.org/10.3168/jds.2013-7733

Silva-del-Rio, N., Rolle, D., Garcia-Munoz, A., Rodriguez-Jimenez, S., Valldecabres, A., Lago, A., & Pandey, P. (2017). Colostrum immunoglobulin G concentration of multiparous Jersey at first and second milking is associated with parity, colostrum yield, and time of first milking, and can be estimated with Brix refractometry. J. Dairy Sci. 100(7), 5774-578. doi: 10.3168/jds.2016-12394.

Soufleri, A., Banos, G., Panousis, N., Fletouris, D., Arsenos, G., & Valergakis, G. E. (2019). Genetic parameters of colostrum traits in Holstein dairy cows. *J. Dairy Sci., 102*, 11225-11232. doi:10.3168/jds.2019-17054

Stieler, A., Bernardo, B.S., & Donovan, G.A. Neutrophil and monocyte function in neonatal dairy calves fed fresh or frozen colostrum. Intern J Appl Res Vet Med. 10(4), 328-334.

Tsuji, S., Hirata, Y., & Mukai, F. (1990). Comparison of lactoferrin content in colostrum between different cattle breeds. *J. Dairy Sci., 73*(1), 125-128. doi:10.3168/jds.S0022-0302(90)78654-7

Tyler JW, Steevens BJ, Hostetler DE, et al. Colostral immunoglobulin concentrations in Holstein and Guernsey cows. Am J Vet Res 1999;60:1136–9

Ulutas, Z., Sahin, A., & Saatci, M. (2008). Genetic parameters of milk yield in Jersey cows. *Journal of Applied Animal Research*, *34*(1), 29-32. doi:10.1080/09712119.2008.9706935

Urie, N.J., Lombard, J.E., Shivley, C.B., Kopral, C.A., Adams, A.E., Earleywine, T.J., Olson, J.D., & Garry, F.B. (2018). Preweaned heifer management on the US dairy operations:

Part 1 Descriptive characteristics of preweaned heifer raising practices. *J. Dairy Sci*. 101(10), 9168-9184. doi:10.3168/jds.2017-14010

USDA:APHIS:VS, National Dairy Heifer Evaluation Project: General Dairy Report. 1992.

Yamauchi K, Wakabayashi H, Shin K, Takase M. (2006).Bovine lactoferrin: benefits and mechanism of action against infections. *Biochem Cell Biol*. 84(3):291-296. doi:10.1139/o06-054

Yang, M., Zou, Y., Wu, Z. H., Li, S. L., & Cao, Z. J. (2014). Colostrum quality affects immune system establishment and intestinal development of neonatal calves. *J. Dairy Sci., 98*, 7153-7163. doi:10.3168/jds.2014-9238

Yaylak, E., Yavuz, M., & Ozkaya, S. (2016). The effects of calving year and parity on colostrum quality of Holstein cows. *Indian J. Anim. Res, 51*(3), 594-598. doi:10.18805/ijar.11470

Zarei, S., Ghorbani, G.R., Khorvash, M., Martin, O., Hossein Mahdavi, A., & Riasi, A. (2017). The impact of season, parity, and volume of colostrum on Holstein dairy cows colostrum composition. *Agricultural Sciences*. 8, 572-581. doi:10.4236/as.2017.87043.

CHAPTER 4: CONCLUSION AND IMPLICATIONS

Our work demonstrated that mode of birth impacted composition of maternal transition and mature milk with subsequent effects on the calf gut microbiome. Actinobacteria abundances appeared to be the most responsive to milk composition changes when compared to other phyla. However, the overall dynamics of the calf rumen microbiome remained relatively stable in response to changes in milk composition at the phyla level. Further studies should focus on impact of milk composition changes on genera of gut bacteria in order to get a more in depth understanding of the dynamics of milk components and the gut microbiome. Additionally, impacts of maternal environment (i.e. season, diet, calving year, dam age…) on physiological mechanisms involved in the biosynthesis of milk components should be further investigated in order to successfully develop better management programs with the goal of producing optimal colostrum.

Moreover, our work showed that colostrum composition is associated with incidence of respiratory illness in calves. Additionally, composition of colostrum was impacted by factors such as season and breed which demonstrates the need for developing better colostrum management practices. Additionally, genetic variance estimates, heritability, and genetic and phenotypic correlations were calculated for colostrum quality traits (i.e. Brix and volume). This showed that quality traits for colostrum are heritable, making them receptive to genetic selection in order to improve breeding programs. Furthermore, genetic correlations of quality traits with test-day components were weak which would allow for selection of optimal colostrum production without drastic effects on compositional traits. In conclusion, further genetic studies should focus on obtaining more records (i.e. Brix, % and colostrum volume) on further generations to determine breeding values for individual animals in order to successfully integrate selection of quality traits into breeding programs.