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The aim of this thesis was to explore the genetics of antibody content in colostrum and newborn calf serum and how they correlate with production and health traits. Results show that these traits are heritable so the occurrence of failure of passive transfer (FPT) can potentially be reduced through selection. Genome-wide association studies identified makers for antibody content in colostrum, calf serum and milk, providing an eventual tool for genomic selection.

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# Genetics of colostrum, milk and serum antibodies in dairy cattle
Implications for health and production

JUAN CORDERO SOLÓRZANO

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## Implications for health and production

Juan Cordero Solórzano

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# Genetics of colostrum, milk, and serum antibodies in dairy cattle

## Implications for health and production

Juan Cordero Solórzano

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submitted in fulfillment of the requirements for the degree of doctor from
**Swedish University of Agricultural Sciences**
by the authority of the Board of the Faculty of Veterinary Medicine and
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# Genetics of colostrum, milk and serum antibodies in dairy cattle. Implications for health and production

## Abstract

Colostrum with sufficient IgG content is essential for the newborn calf, as it requires this passive immunity to survive during its rearing. Failure of passive transfer (FPT) occurs when a calf does not absorb enough antibodies (<10 g/L of IgG in serum) from the colostrum, which besides timely access to colostrum, can be due to low IgG production of the mother or poor IgG absorption of the calf. The aim of this thesis was to explore the genetics of antibody content in colostrum and newborn calf serum and how they correlate with production and health traits. For papers I, II and III, Swedish Red and Swedish Holstein animals from three experimental farms were studied. Colostrum samples from 1313 cows calving from January 2015 to April 2017 were collected. For two of the farms, serum samples from 868 newborn calves were collected at 1 to 12 days after birth. In paper I, genetic parameters were estimated for antibody traits (total IgG and natural antibodies (NAb)) and indicators (Brix and Serum Total Protein) in colostrum and calf serum. Colostrum traits had heritabilities ranging from 0.16 to 0.31 with repeatabilities from 0.21 to 0.55. Brix had positive genetic correlations with all the other colostrum traits including total IgG (0.68). Genetic correlations with milk yield, protein and fat were non-significant. A negative genetic correlation was observed for Brix and IgG traits with Lactation Average Somatic Cell Score (LASCS), but it was also non-significant. Calf serum traits had heritabilities from 0.25 to 0.59, with a significant maternal effect accounting for 17 to 27% of the variance. Genetic correlations of calf serum traits and calf health for the first three months of life had a negative tendency, but were non-significant. LASCS for the first lactation of the animals studied as calves was negatively genetically correlated with 3 NAb traits. In papers II and III, we performed Genome-wide association studies (GWAS) using an imputed 50K SNP array on colostrum and calf serum. For colostrum (Paper II) genomic regions were found for Brix, total IgG and NAbs, with candidate genes related to immunity. Similarly, calf serum GWAS (Paper III) revealed QTLs for S-IgG, IgM and IgG NAbs with genes linked to molecule transport, gastric acid and salivary secretion, among others. For paper IV, 1,695 milk samples of Holstein Friesian from Dutch herds were analyzed for 16 different NAb traits. GWAS were performed using imputed 777K SNP genotypes. For IgM NAb, significant associations were found with candidate genes related to immunoglobulin structure and early B cell development. We have shown that antibodies in colostrum, milk and serum have an important genetic component and we can pinpoint genomic regions that influence these antibodies. Our results suggest that these traits can potentially provide a tool to reduce FPT using genetic selection.

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# Genetiken bakom råmjölk, mjölk och serum-antikroppar i mjölkkor. Betydelse för hälsa och produktion

## Sammanfattning

Råmjölk med en tillräcklig hög halt av antikroppen IgG är av grundläggande vikt för en nyfödd kalv, eftersom den behöver den här typen av passiv immunitet innan avvänjning. Brister i råmjölksupptag (failure of passive transfer – FPT) uppstår när kalven inte kan absorbera tillräckligt mycket antikroppar (<10 g/L of IgG in serum) ifrån råmjölken. Avhandlingens syfte är att utforska genetiken bakom antikroppshalten i råmjölk och serum från nyfödda kalvar samt att undersöka hur detta korrelerar med egenskaper för produktion och hälsa. I studie I, II och III studerades Svenska röda boskap och Svenska Holstein ifrån tre försöksgårdar. Råmjölksprover från 1313 kor som kalvade mellan januari 2015 och april 2017 samlades. Vid två av gårdarna samlades även serumprover från 868 kalvar mellan en och tolv dagar efter födseln. I studie I uppskattades genetiska parametrar för egenskaper av antikroppar (total IgG och naturliga antikroppar (NAb)) samt indikatorer (Brix och Serum Total Protein). Råmjölkens egenskaper visade en ärftlighet mellan 0.16 och 0.31 med en reproducerbarhet mellan 0.21 och 0.55. Brix visade positiva genetiska korrelationer med alla andra egenskaper för råmjölken, inklusive total IgG (0.68). Korrelationer med mjölkavkastning, protein och fett var inte signifikanta. En negativ, men inte signifikant korrelation observerades för Brix- and IgG-egenskaper med Lactation Average Somatic Cell Score (LASCS). Kalvserumets egenskaper visade en ärftlighet mellan 0.35 och 0.59, med en signifikant effekt på mödernet som bidrar med 17 – 27 procent av skillnaden. Genetiska korrelationer mellan kalvserumets egenskaper och hälsa under dem första tre månaderna visade en negativ trend, men dessa var inte signifikanta. LASCS för kalvarnas första laktation korrelerade negativt med tre egenskaper för naturliga antikroppar (NAb). I studier II och III genomförde vi helgenoms-associationsstudier (genome-wide association studies – GWAS) genom att använda en imputerad 50K SNP array för råmjölk och kalvserum. För råmjölk (studie II) hittade vi genomiska regioner för Brix, total IgG och NAbs, tillsammans med kandidatgener relaterad till immunitet. På ett liknande sätt avslöjade en GWAS på kalvserum (studie III) QTLs för S-IgG, IgM och IgG NAbs med gener som är länkade till molekyltransport, magsyra och utsöndring av saliv. I studie IV analyserades 1,695 mjölkprover från Holstein-Frisisk nötboskap för 16 olika egenskaper av naturliga antikroppar (NAb). GWAS utfördes genom att använda imputerad 777K SNP genotyper. För NAb av typen IgM hittade vi signifikanta associationer till kandidatgener relaterade till immunoglobulinstruktur och tidig B-cells utveckling. Vi har visat att antikroppar i råmjölk, mjölk och serum har en viktig genetisk komponent och vi kan precisera genomiska regioner som påverkar dessa antikroppar. Våra resultat tyder på att dessa egenskaper kan potentiellt användas som ett verktyg för att reducera brister i råmjölksupptag (FTP) hos kalvar genom att använda genetisk selektion.

*Nyckelord:* mjölkkor, råmjölk, serum, mjölk, IgG, naturliga antikroppar, kalvar, brister i råmjölksupptag, helgenoms-associationsstudier

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# Genetica van antilichamen in biest, melk en serum bij melkvee. Gevolgen voor de gezondheid en productie

## Samenvatting

Biest met een voldoende hoog IgG-gehalte is onmisbaar voor het pasgeboren kalf. Het heeft deze passieve immuniteit namelijk nodig om te overleven tijdens de opfok. De passieve overdracht mislukt (failure of passive transfer, FPT) als een kalf niet voldoende antilichamen opneemt (<10 g/l IgG in het serum) uit de biest. Dit kan komen door het IgG-gehalte van de biest of de IgG-opname door het kalf. Dit promotieonderzoek was gericht op de genetische aspecten van het antilichamengehalte in biest en in serum van pasgeboren kalveren, en de correlatie daarvan met productie- en gezondheidskenmerken. In onderzoek I, II en III is gekeken naar Zweedse roodbont en Zweedse Holsteinrunderen op drie proefboerderijen. Er zijn biestmonsters verzameld van 1313 koeien die kalfden tussen januari 2015 en april 2017. Op twee van de boerderijen werden serummonsters verzameld bij 868 kalveren, 1 tot 12 dagen na hun geboorte. In onderzoek I werden de genetische parameters geschat voor biest (Brix-waarde, totaal IgG en natuurlijke antilichamen (NAb)) en kalfsserum (totaal serumeiwit, totaal IgG en NAb). De erfelijkheidsgraad van biestkenmerken varieerde van 0,16 tot 0,31 met een herhaalbaarheid van 0,21 tot 0,55. De Brix-waarde was positief genetisch gecorreleerd met alle andere biestkenmerken, inclusief totaal IgG (0,68). Er waren geen significante genetische correlaties met melkproductie, -eiwit en -vet. Er werd een negatieve genetische correlatie gevonden tussen Brix- en IgG-kenmerken en het gemiddelde celgetal gedurende de lactatie (*Lactation Average Somatic Cell Score*, LASCS), maar die was ook niet significant. De erfelijkheidsgraad van kalfsserumkenmerken lag tussen 0,25 en 0,59, met een significant maternaal effect dat 17 tot 27 procent van de variantie bepaalde. Er was sprake van licht negatieve genetische correlaties tussen kalfsserumkenmerken en gezondheid in de eerste drie levensmaanden, maar deze waren niet significant. LASCS bij de eerste lactatie was negatief genetisch gecorreleerd met 3 NAb-kenmerken. In onderzoek II en III hebben we genoombrede associatiestudies uitgevoerd (genome-wide association studies, GWAS) met behulp van een geïmputeerde (voorspelde) 50K SNP-array op biest en kalfsserum. Voor biest (onderzoek II) werden genoomregio's gevonden voor de Brix-waarde, totaal IgG en NAb's, met kandidaatgenen die verband houden met immuniteit. Op vergelijkbare wijze kwamen uit kalfsserum-GWAS (onderzoek III) QTL's naar voren voor S-IgG-, IgM- en IgG-NAb's met genen die gekoppeld zijn aan onder andere moleculair transport, maagzuur- en speekselafscheiding. Bij onderzoek IV werden 1695 Holstein-Friesian-melkmonsters geanalyseerd op 16 verschillende NAb-kenmerken. Er werd GWAS-onderzoek uitgevoerd met behulp van geïmputeerde 777K SNP-genotypen. Voor IgM-NAb zijn significante associaties gevonden met kandidaatgenen die verband houden met de immunoglobulinestructuur en de vroege ontwikkeling van B-cellen. We hebben aangetoond dat er een belangrijke genetische component is voor antilichamen in biest, melk en serum, en we weten welke genoomregio's invloed hebben op deze antilichamen. Onze resultaten duiden erop dat deze kenmerken mogelijk kunnen dienen als hulpmiddel om FPT te verminderen door genetische selectie

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# Dedication

To science, the imperfect flashlight helping us find black cats in an infinitely large dark room

*"Nobody listens to mathematicians"*
    -Carl Sagan

# Contents

# List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I   Cordero-Solorzano, J., Wensman, J.J., Parmentier H.K., Arts, J.A.J., Tråvén, M., Bovenhuis, H. and de Koning, D.J. Genetic parameters of colostrum and calf serum antibodies in Swedish dairy cattle. (*manuscript*)

II  Cordero-Solorzano, J., Wensman, J.J., Parmentier H.K., Arts, J.A.J., Tråvén, M., Bovenhuis, H. and de Koning, D.J. Total IgG and natural antibodies in colostrum of Swedish dairy cows: a genome-wide association study. (*manuscript*)

III Cordero-Solorzano, J., Wensman, J.J., Parmentier H.K., Arts, J.A.J., Tråvén, M., Bovenhuis, H. and de Koning, D.J. Genomic regions associated with antibody uptake in newborn calves. (*manuscript*)

IV  Cordero-Solorzano, J., Parmentier H.K., Arts, J.A.J., van der Poel, J., de Koning, D.J. and Bovenhuis, H.* (2019). Genome-wide association study identifies loci influencing natural antibody titers in milk of Dutch Holstein-Friesian cattle. *Journal of Dairy Science*. 102(12), pp. 11092-11103

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* Corresponding author

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# Abbreviations

| | |
|---|---|
| ADG | Average daily gain |
| AFC | Age of first calving |
| bp | Base pairs |
| CRB | Swedish Red and Swedish Holstein crossbreds |
| EGF | Epidermal growth factor |
| ELISA | Enzyme-linked immunosorbent assay |
| FDR | False discovery rate |
| FPT | Failure of passive transfer |
| GWAS | Genome-wide association study |
| HRP | Horseradish peroxidase |
| HWE | Hardy-Weinberg Equilibrium |
| Ig | Immunoglobulin |
| IGF | Insulin-like growth factor |
| IL | Interleukin |
| INFγ | Interferon gamma |
| KLH | Keyhole limpet hemocyanin |
| LASCS | Lactation-average Somatic Cell Score |
| LPS | Lipopolysaccharides |
| LTA | Lipoteichoic acid |
| MAF | Minor allele frequency |
| MDP | Muramyl dipeptide |
| miRNA | microRNA |
| NAb | Natural antibodies |
| NAV | Nordic Cattle Genetic Evaluation |
| NF-κB | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| OD | Optical density |
| PGN | Peptidoglycan |
| QTL | Quantitative trait locus |
| RID | Radial immunodiffusion |
| SCC | Somatic Cell Count |
| SLB | Swedish Holstein |
| SRB | Swedish Red |
| STP | Serum total protein |
| Th | T helper cell |
| TNFα | Tumor necrosis factor alpha |
| TS | Total solids |

# 1 Introduction

Rearing heifer calves that are healthy is very important since they comprise future replacements for the milking cows in a dairy herd. They should be raised optimally to reduce the number of dead and sick animals, which cuts veterinary costs and avoids loss of production later in life (Heinrichs and Heinrichs, 2011). Morbidity and mortality are important components of the farm's welfare and indicators of herd health (Ortiz-Pelaez et al., 2008). Average neonatal calf mortality rates range from 5 to 11% in North America and Europe (Brickell et al., 2009; Compton et al., 2017). In Sweden neonate calf mortality (1 to 60 days old) was 3.9% for the 2017/2018 period (Växa, 2019). Given their importance for the farm, efforts should be made to improve calves' health and overall wellbeing.

The first weeks of a calf's life are critical, infectious diseases are very prevalent in young calves before 90 days of age (Svensson et al., 2003). The most frequent diseases during this period are diarrhea (scours) and pneumonia, where diarrhea is the most important for calves less than 30 days of age and pneumonia for heifers over 30 days of age (Svensson et al., 2006). Both of these diseases are caused by different pathogens including bacteria, viruses and parasites (Autio et al., 2007). Farms with high prevalence of calf diarrhea and pneumonia face challenges like economic losses from higher mortalities and increased costs from purchasing replacement heifers to make up for the lost ones (Closs and Dechow, 2017). Several factors are involved in the occurrence of these diseases and transfer of passive immunity from colostrum in newborn calves plays an important role (Dewell et al., 2006).

Calves are born almost agammaglobulinemic (Hasselquist and Nilsson, 2009), meaning that they have a very low level of antibodies at birth (Mayasari et al., 2016). The concentration of antibodies in newborn calves is very low because ruminants have an epitheliochorial placenta made up of six anatomical barriers that prevent the transfer of large molecules between the mother and the calf's bloodstreams (Carter and Enders, 2004). So at birth, calves are highly

dependent on the absorption of maternal antibodies to acquire passive humoral immunity and local protection of the digestive tract. If not enough antibodies are transferred, failure of passive transfer (**FPT**) occurs, defined as < 10 g/L of IgG or < 5.5 g/dL of serum total protein (**STP**) in calf serum at 24 hours after birth (Weaver et al., 2000). Calves with FPT have twice the risk of morbidity or death at a young age compared to calves with proper serum IgG (**S-IgG**) levels (Raboisson et al., 2016).

## 1.1  Colostrum

### 1.1.1 Colostrum composition

Colostrum is defined as a yellowish, thick and viscous liquid, resulting from the first secretion of the mammary gland after parturition (Madsen et al., 2004). It is an essential immune and nutritional resource for the calf as it contains more proteins, vitamins and minerals than milk. Most proteins present in colostrum are immunoglobulins (**Ig**), making up for 70-80%, as opposed to milk, where they only comprise 1-2% (McGrath et al., 2016). Among the immunoglobulins, IgG is the most abundant as it represents about 80% of the Ig followed by IgM (11%) and IgA (9%) (Butler and Kehrli, 2005). These molecules have specific roles in immunity. In general, IgG neutralizes antigens in the blood, while IgA prevents microorganisms from adhering to the surface of mucosal cells, blocking their access to tissues or the bloodstream (Cakebread et al., 2015). In mice, it has been shown that maternally acquired, commensal-specific IgG antibodies coordinate with IgA to limit mucosal T cell responses and reinforce intestinal immunity in neonates (Koch et al., 2016).

Immune-modulating molecules such as growth factors and cytokines, are also present in colostrum. The most abundant growth factors in colostrum and milk are epidermal growth factor (**EGF**), insulin-like growth factor I (**IGF**-I) and IGF-II. Cytokines include several interleukins (**IL**), tumor necrosis factor alpha (**TNFα**) and interferon gamma (**INFγ**) (McGrath et al., 2016). Colostrum also contains maternal leukocytes (macrophages, B and T lymphocytes), playing a role in the regulation of immunity of the calf. Bioactive substances with antimicrobial properties such as lysozyme and lactoferrin are present in colostrum as well (Kehoe et al., 2007). Recently, microRNAs (**miRNAs**) have been described in colostrum, suggesting that they play a key role in the communication between mother and offspring (Van Hese et al., 2020).

### 1.1.2 Colostrum quality

The quality of colostrum is mainly defined by its IgG concentration and there is large variation of this trait among individuals (Genc and Coban, 2017). Colostrum should contain at least 50 g/L of IgG to be considered of sufficient quality to transfer passive immunity to the calf (Besser et al., 1991). IgG content is influenced by several factors such as time of sampling after calving (Conneely et al., 2013), parity (Silva-del-Río et al., 2017), nutrition (Nowak et al., 2012), season (Morin et al., 2001), heat stress (Nardone et al., 1997) and dry period length (Mayasari et al., 2015) among others.

The concentration of IgG declines rapidly postpartum; Moore et al. (2005) found that IgG had nearly halved in colostrum 14 hours after birth. Colostrum collected within 1-2 hours postpartum contains the highest amount of IgG and ideally it should be collected with a maximum delay of 6 h postpartum to minimize IgG loss (Godden, 2008).

Parity is known to influence colostrum IgG. In general, colostrum from animals on their first or second parity have similar levels, but after the third or fourth lactation, the IgG levels rise consistently (Weaver et al., 2000; Conneely et al., 2013).

Significant differences of colostrum IgG have been shown between breeds (Weaver et al., 2000). In Sweden, the most common breeds of dairy cattle, Swedish Red (**SRB**) and Swedish Holstein (**SLB**) have been studied as well. Liberg (2000) compared colostrum quality between SRB and SLB but found no significant difference between them.

## 1.2  Passive immunity of the calf

Passive transfer is the process of absorption of maternal Ig from colostrum across the small intestine during a short period of time after birth. The calf receives an effective passive immunity when it absorbs a sufficient amount of Ig from the colostrum, usually defined as more than 10 g/L of IgG in serum (Reschke et al., 2017). A peak of colostrum IgG in the calf serum can be noticed about 24 to 48 hours after the first ingestion of colostrum (Wilm et al., 2018).

### 1.2.1 Failure of passive transfer (FPT)

If not enough antibodies are absorbed by the calf, the result is FPT. This phenomenon is not a disease per se but the calf is more prone to infections, increasing the risk of morbidity and mortality. The majority of diseases in calves are infectious, so an adequate transfer of maternal antibodies is critical. For FPT calves, they must invest energy in producing an immune response, energy that

could otherwise be used for growth (Nonnecke et al., 2012). The gastrointestinal tract of neonatal animals produce limited gastric acid or digestive enzymes. This protects Ig from degradation, but also makes the animal more susceptible to bacterial contamination of colostrum (Baintner, 2007).

The prevalence of FPT in Swedish dairy farms ranges from 14% (Liberg, 2000) up to 40% (Hertel, 2012) according to previous studies. In US farms it has been estimated to be 20% (Beam et al., 2009), 37% in Canadian farms (Trotz-Williams et al., 2008) and 35% in Czech Republic (Staněk et al., 2019).

### 1.2.2 Factors involved in passive immunity transfer

There are different reasons why a calf might not reach satisfactory antibody levels in serum. One of those is colostrum quality, which is affected by several factors mentioned in section 1.1.2. If there is not enough IgG in colostrum, the calf will not absorb enough antibodies.

On par with colostrum quality is the volume of first meal (first colostrum given to the calf). The volume of colostrum required will depend on the IgG concentration in colostrum; it is generally advised that the calf should ingest at least 100 g of IgG (Davis and Drackley, 1998). In a study by Besser et al. (1991) they observed that only about a third of their colostrum samples were above 50 g/L, so feeding the animals just 2 L would not be enough. They estimated that about 85% of their samples would provide the required 100 g of IgG if 4 L were fed. Morin et al. (2010) found that mean serum IgG at 24 hours was significantly higher for calves fed 4 L of colostrum at 0 hours and a further 2 L at 12 hours (31.1 mg/mL IgG) as opposed to calves fed only 2 L at 0 hours and then 2 L at 12 hours (23.5 mg/mL).

Time of first meal, defined as the time after birth when the calf is fed its first colostrum dose, is quite important. During the first 24 hours of life, the neonatal ruminant has the temporary ability to absorb macromolecules such as immunoglobulins in the intestine. At a certain time after birth, this route of macromolecules absorption is terminated and the gut "closes" by a mechanism not fully understood yet (Weaver et al., 2000). The capacity of absorption has been reported to peak during the first 4 hours of life and then slowly decreases until 12 hours where it declines rapidly (Osaka et al., 2014).

### 1.2.3 Evaluating passive immunity transfer

To assess if the calf has enough antibodies in serum after colostrum intake, IgG concentration must be measured in animals from 1 to 7 days old, before the calf's immune system starts producing its own antibodies (Godden, 2008). Most of

these methods are also applied to evaluate colostrum quality (IgG). There are different direct and indirect methods available for this purpose.

Radial immunodiffusion (**RID**) is the gold standard for measuring IgG directly in colostrum or blood and is used as reference for other methods (Bielmann et al., 2010). This method requires an adequate laboratory setting, is time-consuming and relatively expensive compared to other methods. Another option for direct measurement of IgG in colostrum or blood is a quantitative enzyme-linked immunosorbent assay (**ELISA**), which is cheaper and marginally faster than RID but also requires an appropriate laboratory setting (Gelsinger et al., 2015). RID and ELISA do not correlate well, the best correlation reported between these methods was in bovine plasma, with a Pearson value of 0.59, where ELISA was found to underestimate IgG compared to RID (Gelsinger et al., 2015).

Passive transfer can be assessed with indirect methods measuring total solid (**TS**) or total protein (TP), usually with a refractometer. By this technique, the refraction of light is measured in serum or colostrum to produce an estimation of total protein concentration. STP has been found to correlate reasonably well (r = 0.72) with IgG measured by RID (Weaver et al., 2000). For STP, usually a cut-off of 5.2 to 5.5 g/dL is used to diagnose FPT (Calloway et al., 2002). A correlation of 0.77 was observed between STP measured using a digital Brix refractometer and RID. Between an optical refractometer and RID it was 0.79 (McCracken et al., 2017). Another study reported a Brix STP and RID correlation as high as 0.93 (Deelen et al., 2014). Brix has also been used to evaluate colostrum quality, with varying degrees of correlation with RID: Quigley et al. (2013) reported a correlation of 0.75, Bielmann et al. (2010) 0.71, whereas Bartier et al. (2015) reported a correlation of 0.64. These studies reported cut off points for colostrum quality at 21 to 22% to approximate 50 g/L of IgG.

RID is considered as reference when evaluating S-IgG because it presents a high accuracy but can only be performed in a laboratory where the environmental conditions are controlled. Refractometer tests have the advantage that they are easier and faster to perform. For serum, since it requires centrifugation to separate the blood, a laboratory is still needed to evaluate FPT. In the case of colostrum, Brix can be adapted to be used in-the farm as routine quality control.

## 1.3  Genetics of quantitative traits

Breeding programs generally look at complex traits that are controlled by many loci (polygenic) where each locus has a small effect on the trait's phenotype

(Goddard and Hayes, 2009). Complex traits must be studied on a population level to be able to measure the environmental effects. Quantitative genetics looks at these phenotypes and how they vary and breaks down this variation into genetic and environmental components, in order to predict the genetic value of the individuals or families studied (Falconer and Mackay, 1996).

The main goal of breeding research is to estimate the genetic variance transmitted to the offspring, that is, the additive genetic variance (breeding value) and the ratio of said variation versus the total phenotypic variance to estimate heritabilities ($h^2$). In general terms, heritability describes the proportion of the variation that is due to genetic variation on a trait and it is specific to the population and environment in which it was estimated (Oldenbroek and van der Waaij, 2015). When $h^2$ is close to zero, it indicates that almost all the variance in that trait is due to environmental factors, meaning that genetic differences between individuals have very little influence on the phenotype. On the contrary, a heritability close to one means that most of the variance comes from genetic differences, with little contribution from the environment (Walsh et al., 2018). The heritability of a trait does not provide information about specific genes or environmental factors involved, this measure helps to understand complex traits with many contributing factors, such as immune response.

An important part of analyzing a trait is to estimate how it relates to other traits. It is possible that two traits are influenced at least partially, by the same locus (or loci) in what is called pleiotropy. The relationship between the genetic variances of two traits is called genetic correlation ($r_g$) (Falconer and Mackay, 1996). When $r_g$ is zero, it implies that the genetic variances between individuals in these two traits are independent from each other, whereas a value of one implies that the genetic variances of these two traits have a strong association. This means that if two traits are positively correlated and one of the traits is increased through selection, the other trait will increase as well. A negative correlation would make one of the traits go up and the other go down.

Historically, in breeding programs selection has been based on phenotypic and additive genetic variances estimated from phenotype records and pedigrees (Walsh et al., 2018). Which has been quite successful, but it can be slow for traits that are measured only on one sex such as milk in dairy cattle or later in life such as carcass weight or longevity. For such kind of traits, identifying genes and directly selecting animals that carry favorable alleles can be valuable (Goddard and Hayes, 2009). In general, two different approaches have been used to find genes and polymorphisms associated to traits. One is to map the genes that affect the trait to a chromosomal location using genetic markers such as single nucleotide polymorphisms (**SNP**) or microsatellites, the other is to target specific

genes based on their known (or potential) role on the trait (for example, mRNA expression) (Raven et al., 2014).

Based on genotypic information of thousands of SNPs from individuals in a population it is possible to perform a genome-wide association study (**GWAS**) to identify genes or regions influencing traits. The results from these analyses can be used for genetic improvement of animals at a much faster rate using genomic selection (Hayes and Goddard, 2010). Genomic selection has been successfully to select dairy cattle doubling the rate of genetic progress for economically important traits, decreased generation interval, increased selection accuracy, reduced previous costs of progeny testing, and allowed identification of recessive lethal alleles (Wiggans et al., 2017).

### 1.3.1 Genetics of antibodies in colostrum and calf serum

There is a large variation in colostrum quality between cows, even from animals of the same farm and breed (Løkke et al., 2016). Some of this variation can be explained by environmental factors, but there is an important genetic component in colostrum antibody content. A previous study estimated a heritability of 0.4 (0.3) for colostrum IgG (Gilbert et al., 1988). More recently, a study by Soufleri et al. (2019) found a heritability of 0.27 (0.09) for Brix % in Holstein cows colostrum. Certain genes have been suggested to have an effect on colostrum antibody content, Berry et al. (2013) found haplotypes of polymeric immunoglobulin receptor gene (*PIGR*) significantly associated with IgA content in colostrum. A GWAS study found one QTL on chromosome 3 associated with Brix % in colostrum (Kiser et al., 2019).

In spite of its importance for calf health, very few studies have focused on the genetics associated with calf antibody uptake from colostrum. It has been observed that even when the time of first meal, volume of first meal, colostrum IgG concentration and other variables have been accounted for, there is still some significant unexplained variation left in calf antibody uptake (Nilsson, 2015) so part of this variation can be due to genetics. Two studies in the 1980s suggest that this could be the case, Gilbert et al. (1988) estimated a heritability of 0.56 (0.25) for calf serum IgG1 in 36 hour-old calves using a paternal half-sib analysis accounting for the dam's colostral IgG1 concentration. On the other hand, Burton et al. (1989) estimated a heritability of 0.18 (0.25) for IgG in calves 24 to 36h old using a paternal half-sib analysis. Further evidence to support the role of genetics in calf antibody uptake comes from Laegreid et al. (2002), who reported haplotypes of bovine neonatal Fc receptor α-chain gene (*FCGRT*) having an effect on neonatal serum IgG concentration.

## 1.4 Natural Antibodies

Natural antibodies (**NAb**) are immunoglobulins produced without any antigenic stimulation (Avrameas, 1991). They provide a first line of defense against pathogens before specific antibodies (**SpAbs**) are produced as part of the adaptive immune response. NAbs have a polyreactive nature, low affinity and target self-antigens and other molecules like keyhole limpet hemocyanin (**KLH**) and pathogen-associated molecular patterns (**PAMPs**) (Baumgarth et al., 2005).

KLH is extracted from the mollusk keyhole limpet *Megathura crenulata*. It is a highly antigenic large molecule (350-400 kDa) that activates the humoral and cellular immune responses even in non-immunized individuals (Harris and Markl, 1999). Relevant microbial PAMPs include lipoteichoic acid (**LTA**), lipopolysaccharide (**LPS**), peptidoglycan (**PGN**) and muramyl dipeptide (**MDP**), all of which are different components of bacterial cell walls of Gram positive and Gram negative bacteria (Hedges et al., 2005).

NAbs in dairy cattle have been associated with risk of mastitis (Thompson-Crispi et al., 2013), length of productive life (de Klerk, 2016), lameness (Denholm et al., 2018) and postpartum uterine health (Machado et al., 2014). In other species like chicken, serum NAbs have been linked to survival (Star et al., 2007) and *E. coli* resistance (Berghof et al., 2019).

Antibodies (immunoglobulins) in general are classified into isotypes like IgM, IgG, IgA and others, based on their heavy chain structure. In early stages of an infection, IgM is the first isotype produced, allowing a quick response to a variety of PAMPs and other antigens (Schroeder and Cavacini, 2010). Upon antigenic stimulation, B cells may switch isotype and convert into plasma cells that produce more specific IgG antibodies (Liljavirta et al., 2014).

An important part of the humoral immune response rests on NAbs, making them an interesting target to potentially select for disease resistance.

# 2 Aims of the thesis

The overall aim of this thesis was to explore the genetics of antibody content in different bodily fluids of dairy cattle and how they correlate with production and health traits. We focused on colostrum and newborn calf serum to investigate if the occurrence of FPT can be reduced through selection.

The specific aims are as follow:

➢ Investigate if there is a measurable genetic component in calf antibody uptake from colostrum using serum antibody traits and STP as an indicator and how these traits correlate with health and milk production later in life (Paper I).

➢ Measure genetic components in colostrum antibody traits and Brix as an indicator and how these traits correlate to milk production and calves' antibody uptake (Paper I).

➢ Search for genomic regions associated with colostrum and calf serum antibody traits to identify possible candidate genes (Papers II and III).

➢ Identify genomic regions associated with different natural antibody traits in milk and potentially describe candidate genes (Paper IV).

# 3   Materials and methods

The samples and data comprising papers I to III come from three experimental farms in Sweden, whereas the data for study IV is from 379 commercial Dutch herds, more detailed descriptions are provided on each paper. An overview of samples and available data for milk production and health used in papers I to III can be found in Figure 1. Additionally, information about the traits used in papers I to III can be seen in Table 1.

## 3.1   Samples

### 3.1.1 Swedish samples (Papers I to III)

The samples were collected at three experimental farms: The Swedish Livestock Research Center Lövsta (Uppsala), Röbäcksdalen forskningsstation SITES (Umeå) and Nötcenter Viken (Falköping) from January 2015 to April 2017. Ethics approval number from the Swedish Board of Agriculture is C 140/14.

The pedigree used for all animals contained 29,048 records (20 generations) and was made available by Växa Sverige.

*Cows*

In total 1,340 cows were sampled, out of which 682 were Swedish Red (**SRB**), 460 Swedish Holstein (**SLB**) and 198 SLB and SRB crossbreds (**CRB**). Parities ranged from 1 to 6 with ~90% of the animals between 1 and 3 parities. During the sampling period 504 cows calved twice and 29 calved three times.

Sampling of first milking colostrum was carried out by staff at the farms, recording the time between birth and sampling for a total of 1711 individual samples. Additionally, milk and serum samples were taken from cows at Lövsta; 290 milk samples between February 2015 to March 2016 at 6 to 24 days in milk

and 330 serum samples from January 2015 to June 2016, 23 to 1 days before calving.

Milk production information was provided by Växa Sverige for 1283 cows, comprising 305-day cumulative average of milk yield (kg), fat and protein percentage and lactation-average somatic cell score (**LASCS**) from lactations matching the corresponding colostrum sample.
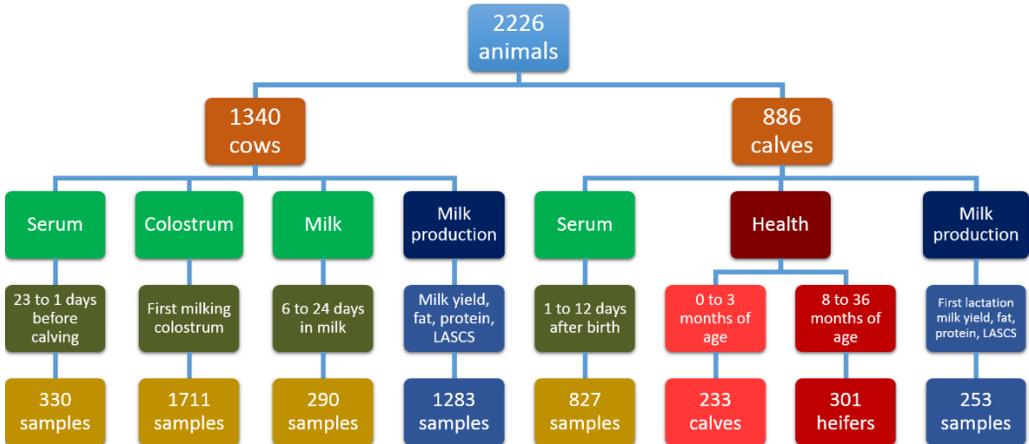


*Figure 1.* Overview of samples and available data for milk production and health (Papers I to III)

## Calves

A total of 886 calves were sampled at Lövsta and Röbäcksdalen, borne by cows that were sampled for colostrum, in order to pair the calves' serum with the colostrum of their dams. However, 59 of those calves had an unknown pedigree, so they were excluded from the study. The remaining 827 calves were 520 SRB, 211 SLB and 96 CRB.

Neonates were separated from the dam as soon as possible after birth. In Lövsta, animals were predominantly fed by nipple bottle and those too weak to suckle were fed by esophageal tube. All calves were given first milking colostrum as the first meal from the mother, except for 119 calves that were fed colostrum from donor cows.

Sampling was carried out by staff at the farms. Blood was drawn using a vacutainer system (BD Biosciences, NJ, USA) for collection of serum, in tubes without additives, and anti-coagulated blood in tubes with $K_3$-ethylenediaminetetraacetic acid (**EDTA**). Anti-coagulated blood was used for DNA extraction and genotyping.

Calves were sampled at the age of 1 to 12 days, but 93% of the samples were taken before 8 days of age. Information for each individual was recorded about

birth weight, volume of first meal, time of first meal after birth and from which cow the colostrum was fed to the calf.

Disease information and treatments during the first three months of age were recorded for 233 calves born in Röbäcksdalen during the sampling period. Additional health information was made available by Växa Sverige for sickness reports from Lövsta and Röbäcksdalen at 8 to 36 months of age. In both cases trauma reports were excluded.

First parity milk production information was provided by Växa Sverige for 253 of the calves included in the project, comprising 305-day cumulative average of milk yield (kg), fat and protein percentage and LASCS.

## 3.1.2 Dutch data (Paper IV)

Milk samples and phenotypes were collected as part of the Dutch Milk Genomics Initiative [for details see Stoop et al. (2008)]. The NAb levels were measured as titers. Phenotypes included in the present study were 1) total NAb titers binding the antigens KLH, LPS, LTA or PGN without isotype distinction and 2) NAb isotypes IgA, IgG1 and IgM titers binding KLH, LPS, LTA and PGN, for a total of 16 traits.

Titers were measured in morning milk samples by an indirect ELISA (Ploegaert et al., 2010) and were available for 1,695 genotyped cows that were at least 87.5% Holstein-Friesian and housed in 379 commercial dairy herds across the Netherlands. The average number of days in lactation when milk samples were taken was 166, with a range from 66 to 263 days.

The pedigree used contained 12,998 animals (4 generations) and was made available by the Dutch cattle cooperative CRV (Arnhem, The Netherlands).

Cows were genotyped for 50,905 SNP with a custom Infinium assay (Illumina, San Diego, CA, USA) designed by CRV. Imputed 777K SNP data from the aforementioned 50K SNP array genotypes were available for 1,736 animals from the Milk Genomics project. Further information about the genotyping and imputation of the samples can be found in Duchemin et al. (2014). SNP positions were mapped using UMD 3.1.1/bosTau8 assembly of the bovine genome (Zimin et al., 2009).

## 3.2  Phenotypes (Papers I to III)

### 3.2.1 Brix percentage as an estimate for colostrum IgG

Colostrum samples were analyzed using a digital refractometer Atago 3810 PAL-1 (Atago Tokyo, Japan) to measure Brix percentage (which approximates total solid (**TS**) percentage) as an estimate for IgG content. A total of 1682 individual samples were analyzed. Each sample was measured three times to average a sample value.

### 3.2.2 Serum total protein (STP)

Optical refractometer AO Veterinary Refractometer 10436 (AO Scientific Instruments, Buffalo NY, US) was used to measure TS in calf serum samples as an estimate of STP (g/dL). A total of 808 samples were analyzed. Each sample was measured three times to average a sample value.

### 3.2.3 Total IgG

*Cows*

Colostrum IgG was measured only in samples with a matching calf serum sample using a commercial ELISA (Bovine IgG ELISA Kit E-10G Immunology Consultants Laboratory Inc, Oregon, US) according to the manufacturer's instructions. Standards and samples were run in duplicates and a four parameter logistic regression was used to calculate IgG concentration (g/L). Colostrum was diluted at 1:400,000 but samples with high values outside the standard reference curve limits were retested at 1:800,000. In total, 686 colostrum samples were analyzed.

*Calves*

Serum IgG concentration was determined using an ELISA kit (Bovine IgG ELISA Quantitation Set E10-118 Bethyl Laboratories Inc, Texas, US) according to the manufacturer's instructions. Standards and samples were run in duplicates and a logit regression was used to calculate IgG concentration (g/L). Serum was diluted at 1:112,000 however, samples with values outside the standard reference curve limits were retested in different dilutions (1:224,000 or 1:16,000 for higher or lower values, respectively). In total, 810 samples were analyzed.

To avoid confusion with the NAb traits, these IgG measurements will be referred to as "colostrum total IgG" or simply "total IgG" for colostrum and "serum IgG" or "S-IgG" for calf serum.

Table 1. *Overview of traits and number of samples by breed and farm (Papers I to III)*

| Sample type | Trait | N | Breed[1] | | | Farm[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | | SRB | SLB | CRB | L | R | V |
| Colostrum | Brix | 1682 | 870 | 567 | 245 | 568 | 225 | 889 |
| | Total IgG | 686 | 482 | 204 | - | 504 | 185 | - |
| | NAb[3] | 1519-1532 | 808 | 515 | 209 | 570 | 228 | 734 |
| First test milk | NAb[3] | 248-272 | 170 | 102 | - | 272 | - | - |
| Pre-parturition serum | NAb[3] | 321 | 197 | 124 | - | 321 | - | - |
| Calf serum | STP | 808 | 507 | 207 | 93 | 581 | 227 | - |
| | S-IgG[4] | 810 | 509 | 210 | 91 | 584 | 226 | - |
| | NAb[3] | 774-827 | 520 | 211 | 96 | 594 | 233 | - |

[1.] SRB = Swedish Red, SLB = Swedish Holstein, CRB = SRB/SLB Crossbred

[2.] L = Lövsta forskningscentrum, R = Röbäcksdalen forskningsstation SITES, V = Nötcenter Viken

[3.] Comprises six traits: KLH-IgA, KLH-IgG, KLH-IgM, MDP-IgA, MDP-IgG and MDP-IgM

[4.] Serum IgG

### 3.2.4 Natural Antibodies (NAb)

Titers for natural antibodies were measured in all sample types for cows (colostrum, first test milk and pre-parturition serum) and calves (serum).

Optical density (OD) of muramyl dipeptide (MDP) and keyhole limpet hemocyanin (KLH)-binding immunoglobulins of the isotypes IgM, IgA and IgG were measured by an indirect two-step ELISA, as outlined by Ploegaert et al., 2010.

Colostrum and serum samples were prediluted at 1:10, whereas milk samples at 1:5, with phosphate-buffered saline containing 0.05% Tween 20 (PBST pH 7.2, dilution buffer). Flat-bottomed, 96-well medium binding plates were coated with 100 μl/well of 2 μg/ml of KLH (Sigma H8283), or MDP (Sigma A9519) respectively, in carbonate buffer (10.6 g/L $Na_2CO_3$, pH 9.6). After incubation overnight at 4°C, plates were washed with tap water containing Tween 20 and blocked with 100 μl/well of 5% normal chicken serum in PBST for one hour at room temperature.

Predilutions were further diluted in the antigen-coated plates with dilution buffer to 1:40, 1:160, 1:640, and 1:2,560 test dilutions for colostrum and serum and 1:10, 1:20, 1:40, and 1:80 for milk. One unrelated colostrum sample was chosen as standard positive to be consistently used in all assays. Duplicates of this standard positive were stepwise 1:1 diluted with dilution buffer (8 serial

dilutions from 1:20 until 1:2,560) and pipetted into the antigen-coated plates. The plates were incubated for one hour and a half at room temperature.

After washing, MDP or KLH plates were incubated with 100 μl/well of either 1:40,000-diluted rabbit antibovine IgM labelled with horseradish peroxidase (HRP) (Bethyl A10-100P), 1:40,000-diluted sheep antibovine IgG HRP (Bethyl A10-118P) or 1:20,000-diluted rabbit antibovine IgA HRP (Bethyl A10-108P) for one hour and a half at room temperature. After washing, 100 μL/well of tetramethylbenzidine (TMB, 71.7 μg/ml) with 0.05% hydrogen peroxide was added and incubated for approximately ten minutes at room temperature. The reaction was stopped with 50 μL of 1.25M $H_2SO_4$. OD was measured with a Multiskan Go (Thermo scientific) at 450 nm.

Antibody titers were calculated as described by Frankena (1987) [cited by de Koning et al. (2015)]. OD of the duplicate standard positive samples were averaged for each plate. Logit values of OD per plate were calculated using:

$$\text{logit OD} = \ln\left(\frac{\text{OD}}{\text{OD}_{max} - \text{OD}}\right)$$

where OD is the OD of a well, and $\text{OD}_{max}$ is the maximum averaged OD of the duplicate standard positive samples. The last positive well (lpw) of the averaged duplicate standard positive was set to the sixth dilution for colostrum and serum and to the seventh for milk. Titers of each sample per plate were calculated using:

$$\text{titer} = \frac{\text{logit OD}_{lpw} - \left(\text{logit OD}_{lpw} - \beta * \log_2(\text{dilution}_{sample})\right)}{\beta}$$

where logit $\text{OD}_{lpw}$ is the estimated logit OD at the lpw calculated with the estimated linear regression function using the $\log_2$-dilution value of that well, logit $\text{OD}_{sample}$ is the logit OD calculated of the OD closest to 50% of $\text{OD}_{max}$ for a sample of an individual ($\text{OD}_{sample}$), $\beta$ is a regression coefficient of the logit OD against the respective $\log_2$-dilution values of the averaged duplicate standard positive samples, and $\log_2$ dilution$_{sample}$ is the $\log_2$-dilution value at which $\text{OD}_{sample}$ occurred, as described by de Koning et al. (2015).

Six traits were generated for each sample type from three isotypes measured for two antigens: KLH-IgA, KLH-IgG, KLH-IgM, MDP-IgA, MDP-IgG and MDP-IgM. A total of 1,510 to 1,532 samples (depending on the trait) were analyzed for each colostrum trait, 248 to 272 for first test milk, 321 for pre-parturition cow serum and 774 to 827 for calf serum. A constant was added to each trait to make all the values above zero.

## 3.3 Genotypes (Papers II and III)

Blood samples without anticoagulant taken from the calves were used for DNA analysis. Some of the animals had already been genotyped as part of the Nordic Cattle Genetic Evaluation (**NAV**) routine, but 445 additional animals not retained for breeding (bull calves and non-replacement heifers) were genotyped. Genotyping was done by Eurofins GenoSkan (Aarhus, Denmark) using BovineLD BeadChip 7K chip assay (Illumina, San Diego, CA, USA), the same chip used for the animals that were already genotyped.

Imputed 50K SNP genotypes were provided by NAV. Imputation was performed only on purebred SRB or SLB individuals using Fimpute v2.2 (Sargolzaei et al., 2014). For SLB, 473 animals were imputed using a reference population of 90,000 individuals resulting in 46,342 imputed SNPs. For SRB, 1,038 animals were imputed using a reference population of 80,000 individuals with 46,912 imputed markers. In total for the 1,511 animals, 45,823 shared markers imputed for both breeds were available. The positions of the imputed SNP were based on the bovine genome assembly UMD 3.1.1 (Zimin et al., 2009). Ultimately, 706 calves and 829 cows from the project had imputed 50K genotypes.

## 3.4 Statistical analysis

Variance components for genetic effects, repeated measurements (colostrum traits) and maternal effects (calf serum) were estimated with animal models using ASReml 4.1 (Gilmour et al., 2015).The same method was used for estimating genetic and phenotypic correlations, and performing Genome-wide association studies.

### 3.4.1 Models (Papers I to III)

*Cows*

For colostrum traits, the following repeatability model was used:

$$y_{ijklm} = \mu + \beta_1 C2CS_{ijklm} + parity_i + breed_j + HYSP_k + A_l + pe_m \\ + e_{ijklm} \quad (1.)$$

where y is the observation of the trait; μ is the overall mean of the trait; $C2CS_{ijklm}$ is a covariate describing the effect of colostrum sampling time after calving in hours; $parity_i$ represents the fixed effect of four parity classes (1, 2, 3 and 4 or more); $breed_j$ is the fixed effect of breed (SLB, SRB or CRB); $HYSP_k$

describes the fixed effect of Herd-Year-Season of calving and sample storage Plate number; $A_l$ the random additive genetic effect assumed to be distributed as $N(0, A\sigma_a^2)$, where $\mathbf{A}$ is the additive genetic relationships matrix from the pedigree and $\sigma_a^2$ is the additive genetic variance; $pe_m$ is the random permanent environment effect assumed to be distributed as $N(0, I\sigma_{pe}^2)$, where $\mathbf{I}$ is the identity matrix and $\sigma_{pe}^2$ is the permanent environment effect variance; $e_{ijklm}$ is the random residual effect assumed to be distributed as $N(0, I\sigma_e^2)$, where $\mathbf{I}$ is the identity matrix and $\sigma_e^2$ is the residual variance.

For first test milk and pre-parturition cow serum NAb traits, the following animal model was used:

$$y_{ijklm} = \mu + \beta_1 C2S_{ijklm} + parity_i + breed_j + YS_k + Plate_l + A_m + e_{ijklm} \qquad (2.)$$

where y is the NAb titer of milk or serum; μ is the overall mean of the trait; $C2S_{ijklm}$ is a covariate describing the effect of sampling time in days before (serum) or after (milk) calving ; $parity_i$ represents the fixed effect of four parity classes (1, 2, 3 and 4 or more); $breed_j$ is the fixed effect of breed (SLB, SRB or CRB); $YS_k$ describes the fixed effect of Year-Season of calving; $Plate_l$ is the fixed effect of sample storage Plate number; $A_m$ the random additive genetic effect assumed to be distributed as $N(0, A\sigma_a^2)$, where $\mathbf{A}$ is the additive genetic relationships matrix from the pedigree and $\sigma_a^2$ is the additive genetic variance; $e_{ijklm}$ is the random residual effect assumed to be distributed as $N(0, I\sigma_e^2)$, where $\mathbf{I}$ is the identity matrix and $\sigma_e^2$ is the residual variance.

*Calves*

For calves' traits, the following animal model was used:

$$y_{ijkl} = \mu + \beta_1 COL_{ijkl} + \beta_2 BLD_{ijkl} + \beta_3 BWT_{ijkl} + breed_i + HYSP_j + A_k + m_l + e_{ijkl} \qquad (3.)$$

where y is the observation of the trait; μ is the overall mean; $COL_{ijkl}$ is a covariate describing the absolute amount of antibodies received from the matching colostrum trait (Brix, colostrum IgG or NAb * volume of first meal); $BLD_{ijkl}$ is a covariate describing the time from calving to blood sampling in days; $BWT_{ijkl}$ is the covariate of calf birth weight in kg; $breed_i$ is the fixed effect of breed (SLB, SRB or CRB); $HYSP_j$ describes the fixed effect of Herd-Year-Season of calving and sample storage Plate number; $A_k$ the random additive genetic effect assumed to be distributed as $N(0, A\sigma_a^2)$, where $\mathbf{A}$ is the additive genetic relationships matrix from the pedigree and $\sigma_a^2$ is the additive genetic variance; $m_l$ is the maternal environment effect assumed to be distributed as $N(0, I\sigma_m^2)$, where $\mathbf{I}$ is

the identity matrix and $\sigma_m^2$ is the maternal variance; $e_{ijkl}$ is the random residual effect assumed to be distributed as $N(\textbf{0, }I\sigma_e^2)$, where $\textbf{I}$ is the identity matrix and $\sigma_e^2$ is the residual variance.

### 3.4.2 Heritabilities and variance proportions (Paper I)

Heritabilities were estimated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2} \tag{4.}$$

with phenotypic variance $\sigma_p^2 = \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2$ for model (1.), $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$ for model (2.) and $\sigma_p^2 = \sigma_a^2 + \sigma_m^2 + \sigma_e^2$ for model (3.), where $\sigma_a^2$ is the additive genetic variance, $\sigma_{pe}^2$ the permanent environment variance, $\sigma_m^2$ is the maternal variance and $\sigma_e^2$ the residual variance.

Repeatability for colostrum traits was:

$$r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2} \tag{5.}$$

where $\sigma_a^2$ is the additive genetic variance, $\sigma_{pe}^2$ the permanent environment variance and $\sigma_e^2$ the residual variance

Maternal contribution or maternal variance proportion for calf traits was estimated as:

$$m^2 = \frac{\sigma_m^2}{\sigma_a^2 + \sigma_m^2 + \sigma_e^2} \tag{6.}$$

Genetic correlations between colostrum traits were estimated using a bivariate analysis of model (1.) and for calf serum traits a bivariate of model (3.) as follows:

$$r_g = \frac{\sigma_{a1,a2}^2}{\sqrt{(\sigma_{a1}^2 * \sigma_{a2}^2)}} \tag{7.}$$

where $\sigma_{a1}^2$ is the additive genetic variance for trait 1, $\sigma_{a2}^2$ the additive genetic variance for trait 2, and $\sigma_{a1,a2}^2$ the additive genetic covariance between traits 1 and 2. The same formula was applied for phenotypic correlations, substituting additive genetic variances and covariance for phenotypic ones.

Genetic and phenotypic correlations were also estimated between colostrum and calf serum traits using a bivariate analysis with fixed effects from model (1.) for colostrum traits and fixed effects from model (2.) for calf traits using cow to estimate animal and permanent environment effects. A similar approach was used to estimate genetic and phenotypic correlations of colostrum traits with first

test milk and pre-parturition cow serum, a bivariate analysis with fixed effects from model (1.) for colostrum traits and fixed effects from models (2.) or (3.) for milk and cow serum traits.

Significance of heritabilities and genetic correlations was checked using a log-likelihood ratio test. For heritabilities, the univariate model was compared to the same model in which the additive genetic variance was fixed at zero:

$$\text{LR} = 2 * (\text{loglik}(m_1) - \text{loglik}(m_0)) \tag{8.}$$

with $\text{loglik}(m_0)$ as the value of the log-likelihood under the null hypothesis with the additive genetic variance fixed at almost zero (0.001) and $\text{loglik}(m_1)$ as the value of log-likelihood under the alternative hypothesis without variance components constrained. Assuming that the likelihood ratio follows a $X^2_{df=1}$ distribution, the variance component was deemed significant if $p < 0.05$. For maternal effects and permanent environment effects, a similar approach was used to compare models with and without a constrained (0.001) variance component. For genetic correlations, models with genetic covariance set to almost zero (0.001) and with unconstrained genetic covariance were used as comparison for testing.

### 3.4.3 Health and production traits analysis (Paper I)

*Cows*

Using available milk production data for 305-day cumulative average of milk yield, fat and protein percentage and LASCS, genetic and phenotypic correlations were estimated between these and colostrum traits.

To estimate variance components on milk production traits for the correlations, the following repeatability model was used:

$$y_{ijklm} = \mu + \beta_1 AFC_{ijklm} + parity_i + breed_j + HYS_k + A_l + pe_m + e_{ijklm} \tag{9.}$$

where y is the observation of the milk trait; $\mu$ is the overall mean; $AFC_{ijklm}$ is a covariate describing the effect of age at first calving in months; $parity_i$ represents the fixed effect of four parity classes (1, 2, 3 and 4 or more); $breed_j$ is the fixed effect of breed (SLB, SRB or CRB); $HYS_k$ describes the fixed effect of Herd-Year-Season of calving; $A_l$ the random additive genetic effect assumed to be distributed as $N(0, A\sigma_a^2)$, where **A** is the additive genetic relationships matrix from the pedigree and $\sigma_a^2$ is the additive genetic variance; $pe_m$ is the random permanent environment effect assumed to be distributed as $N(0, I\sigma_{pe}^2)$, where **I** is the identity matrix and $\sigma_{pe}^2$ is the permanent environment effect

variance; $e_{ijklm}$ is the random residual effect assumed to be distributed as $N(0, I\sigma_e^2)$, where $\mathbf{I}$ is the identity matrix and $\sigma_e^2$ is the residual variance.

A bivariate analysis with model (1.) for colostrum traits and model (9.) for production traits was used to estimate the phenotypic and genetic correlations according to formula (7.).

*Calves*

First parity milk production data from 253 of the animals of which serum sample was analyzed when they were calves, was combined with 700 records of first parity milk data from the project calves' dams. Genetic and phenotypic correlations were estimated between calf serum traits and milk production. A bivariate analysis with model (3.) for serum traits and a modified model (9.) removing the fixed effect of parity and the variance component for permanent environment, was used for production traits and age of first calving (**AFC**) to estimate the correlations according to formula (7.).

Disease data for Röbäcksdalen calves (0 to 3 months of age) was arranged as binary (0 = healthy/untreated and 1 = disease/treated) with most of the diseased cases treated with antibiotics. In total, 63 out of the 231 animals were diagnosed with some ailment and received treatment. A bivariate analysis with model (3.) for serum traits and a model including only Year-Season of birth as a fixed effect for health trait, was used to estimate the correlations according to formula (7.).

Health information for cows between 8 to 36 months of age in Lövsta and Röbäcksdalen included 66 animals with reported cases out of the 301 heifer calves from our samples that were kept for milk production. Data was arranged as binary (0 = healthy and 1 = disease) regardless of the condition. A bivariate analysis with model (3.) for serum traits and a model including only Herd-Year-Season of birth as a fixed effect for health trait, was used to estimate the correlations according to formula (7.).

### 3.4.4 Genome-wide association studies (Papers II, III and IV)

*Paper II*

For colostrum, a 50K SNP GWAS was performed on 8 traits: Brix, total IgG and 6 NAbs. Model (1.) including a fixed effect of SNP was used for the analysis.

*Paper III*

A 50K SNP GWAS was performed on calf traits which included STP, S-IgG and 6 NAb traits. The analysis was run using model (3.) including a fixed effect for the SNP.

*Paper IV*

For the Dutch data, the GWAS were performed using the following animal model:

$$y_{ijklmno} = \mu + \beta_1 dim_{ijklmno} + \beta_2 e^{-0.05*dim_{ijklmno}} + \beta_3 ca_{ijklmno} + \beta_4 ca^2_{ijklmno} + season_k + scode_l + SNP_m + herd_n + A_o + e_{ijklmno}$$ (10.)

where y is the observation of the NAb titer; μ is the overall mean of the trait; $dim_{ijklmno}$ is a covariate describing the effect of days in lactation, modeled with a Wilmink curve (Wilmink, 1987); $ca_{ijklmno}$ is a covariate accounting for the effect of age at first calving, $season_k$ is the fixed effect with 3 classes of calving season (June–August 2004, September–November 2004, and December 2004– February 2005); $scode_l$ is the fixed effect of sire type in three classes: proven bull, young bull or other proven bull; $A_o$ is the random additive genetic effect assumed to be distributed as *N(0, $A\sigma^2_a$)*, where **A** is the additive genetic relationships matrix from the pedigree, and $\sigma^2_a$ is the additive genetic variance; $SNP_m$ is the fixed effect of SNP genotype; $herd_n$ is the random herd effect assumed to be distributed as *N(0, $I\sigma^2_{herd}$)*, where **I** is the identity matrix, and $\sigma^2_{herd}$ is the herd variance; $e_{ijklmno}$ is the random residual effect assumed to be distributed as *N(0, $I\sigma^2_e$)*, where **I** is the identity matrix, and $\sigma^2_e$ is the residual variance.

The additive genetic variance and the herd variance were fixed at the values calculated from analyses based on model (10.) without the inclusion of SNP effects. The additive genetic variance, herd variance and other genetic parameters were previously reported by Wijga et al. (2013).

*Quality control (Papers II, III and IV)*

For quality control, monomorphic SNPs were removed and SNPs with genotype classes of 1 to 10 animals were excluded from the GWAS. SNPs with a strong deviation from Hardy-Weinberg Equilibrium (**HWE**) ($X^2$ values ≥ 600) were also excluded. Regarding significant SNPs, the sensitivity of association was evaluated if they had a genotype class with less than 50 animals. In those cases, phenotypic records of animals with that specific genotype class were removed

and the SNP retested to confirm the significance of the association. Furthermore, significant SNPs were inspected for extreme phenotypes. Phenotypes were regarded as extreme when their residuals (from their respective trait model) were larger than ±3.5 standard deviations. If extreme phenotypes were found, the SNP was retested without those extreme phenotypes.

For paper IV, genomic inflation factor ($\lambda$) was accounted for using genomic-control correction dividing the F test statistics by $\lambda$, and recalculating the *p-values* (Devlin et al., 2004).

To control the number of false positives due to multiple testing, a false discovery rate (**FDR**) threshold using the R package "qvalue" (Storey et al., 2015) was estimated. SNP associations were considered suggestive if $0.05<FDR<0.20$ and significant if $FDR<0.05$. Suggestive and significant associations were grouped into genomic regions; SNPs were considered in the same region based on two criteria: 1) they were less than 200kb apart, where linkage disequilibrium's (LD) $r^2$ value is assumed to decrease approximately to 0.15 (de Roos et al., 2008; Khatkar et al., 2008) or 2) fitting two of them simultaneously in the model reduced their significance, suggesting some degree of LD.

### 3.4.5 Candidate genes (Papers II, III and IV)

For the regions associated with a trait, we searched for genes within those regions in NCBI (NCBI Resource Coordinators, 2018) and Ensembl (Zerbino et al., 2018) databases. We used the bovine UMD 3.1.1 assembly for the GWAS SNP mapping.

After gathering the genes in each region, we analyzed them through WebGestalt (Wang et al., 2017) a functional enrichment analysis web tool, to determine which biological pathways they were related to. The search within WebGestalt included 4 databases: KEGG (Kanehisa et al., 2017), PANTHER (Mi et al., 2017), Reactome (Fabregat et al., 2018) and WikiPathways (Slenter et al., 2018).

# 4 Summary of results

## 4.1 Genetic parameters of colostrum and calf serum antibodies in Swedish dairy cows (Paper I)

In this study, we estimated variance components and correlations for different traits in colostrum, first test milk, pre-parturition serum and calf serum. An overview of the traits and genetic parameters for calf traits can be seen in Table 2, and for cows in Table 3.

For calf traits, heritability estimates were moderate to high, ranging from 0.25 to 0.59, except for STP, which was not significant. Maternal contribution was moderate (0.17 to 0.27), with the exception of KLH-IgA. Breed was only significant for STP with an effect size of 0.27 (0.10) for SLB relative to SRB.

Heritability estimates for colostrum traits were moderate, ranging from 0.16 to 0.31 and repeatabilities were moderate to high (0.21 to 0.55). Breed effects were significant and consistently higher for SLB and CRB relative to SRB. The effect of breed varied depending on the trait, but the difference between SRB and SLB was about half a standard deviation in most cases. In the case of first test milk, heritability estimates were not significant except for KLH-IgG with a moderate value (0.33). Breed effects however, were significant for all but one trait, with the difference being approximately one third of a standard deviation. Heritabilities for pre-parturition serum were moderate to high (0.37 to 0.64), except for MDP-IgG which was not significant. Holsteins had a significantly higher effect on three traits; KLH-IgG, MDP-IgG and KLH-IgM.

Table 2. *Descriptive statistics, heritabilities ($h^2$), maternal effects ($m^2$), phenotypic variances ($\sigma_p^2$), breed effects and number of samples for calf serum traits. SE in parenthesis*

| Trait[1] | Mean (SD) | Range[2] | $h^2$ | $m^2$ | $\sigma_p^2$ | Breed[3] SLB | CRB | N |
|---|---|---|---|---|---|---|---|---|
| STP (g/dL) | 6.0 (0.7) | 4.9 – 7.2 | 0.07 (0.11) | **0.22 (0.08)** | 0.44 (0.03) | **0.27 (0.10)** | **0.18 (0.09)** | 808 |
| S-IgG (g/L)[4] | 22.8 (12.8) | 5.6 – 48.7 | **0.25 (0.13)** | **0.27 (0.08)** | 1.28 (0.08) | 0.04 (0.24) | 0.06 (0.18) | 810 |
| KLH-IgA | 4.0 (1.3) | 1.8 – 6.2 | **0.46 (0.14)** | 0.03 (0.08) | 0.85 (0.05) | 0.06 (0.24) | -0.01 (0.16) | 827 |
| KLH-IgG | 9.3 (1.7) | 6.3 – 11.6 | **0.26 (0.14)** | **0.37 (0.08)** | 1.57 (0.10) | 0.19 (0.27) | -0.21 (0.19) | 827 |
| KLH-IgM | 6.1 (1.7) | 3.1 – 8.8 | **0.23 (0.13)** | **0.23 (0.08)** | 2.24 (0.13) | 0.04 (0.31) | -0.04 (0.23) | 827 |
| MDP-IgA | 5.8 (1.8) | 2.6 – 8.4 | **0.43 (0.16)** | **0.17 (0.08)** | 1.63 (0.10) | -0.03 (0.33) | 0.16 (0.22) | 774 |
| MDP-IgG | 6.7 (1.4) | 4.3 – 9.0 | **0.24 (0.14)** | **0.25 (0.08)** | 1.33 (0.08) | 0.14 (0.25) | -0.10 (0.18) | 821 |
| MDP-IgM | 7.8 (1.8) | 4.4 – 10.0 | **0.59 (0.16)** | **0.19 (0.08)** | 1.61 (0.10) | 0.08 (0.35) | 0.03 (0.22) | 825 |

[1.] Significant $h^2$, $m^2$ and breed effects are highlighted in bold
[2.] Range shows 5th quantile and 95th quantile, respectively
[3.] Adjusted breed effects are compared to Swedish Red (SRB)
[4.] Variance components and effects were estimated using a square root transformation

Fixed effects in models (1.) and (3.) were selected based on their significance by an incremental Wald F statistics test including interactions. One factor that was tested and not selected was ELISA plate, which was significant by itself, but when the storage plate effect was added it was no longer significant. This was observed both for colostrum and calf serum traits.

For calf serum, tested factors that were not significant include sex, ease of calving, time of first meal and whether the calf was fed with the mother's colostrum or with colostrum from a donor cow (as logical variable). Volume of first meal and concentration or titer of colostrum fed was combined as a single "absolute fed colostrum" factor with a stronger effect. Figure 2 shows the plots of each calf serum trait versus its matching absolute colostrum trait. Correlation plots show a fairly linear relationship between traits that seems marginally higher for Brix and IgG (0.42 to 0.49) compared to IgM and IgA (0.26 to 0.37).

Time of calving to blood sampling (calves) had varying effects depending on the trait. Figure 3 shows plots for each trait versus the time of blood sampling in days. As opposed to the previous trait, it appears that STP and IgG traits have a slightly lower correlation (−0.09 to −0.21) compared to IgM and IgA (−0.27 to −0.51).

Table 3. *Descriptive statistics, heritabilities ($h^2$), repeatabilities ($r$), phenotypic variances ($\sigma_p^2$), breed effects and number of samples for cow sample type and traits. SE in parenthesis*

| Trait[1] | Mean (SD) | Range[2] | $h^2$ | $r$ | $\sigma_p^2$ | Breed[3] | | N |
|---|---|---|---|---|---|---|---|---|
| | | | | | | SLB | CRB | |
| *Colostrum* | | | | | | | | |
| Brix (%) | 21.9 (4.2) | 14.5 – 28.7 | **0.31 (0.06)** | **0.35 (0.04)** | 15.47 (0.59) | **2.02 (0.82)** | **0.86 (0.52)** | 1682 |
| Total IgG (g/L)[4] | 56.8 (26.9) | 19.7 – 106.6 | **0.20 (0.09)** | **0.21 (0.08)** | 2.49 (0.14) | **0.68 (0.32)** | - | 686 |
| KLH-IgA | 5.9 (1.3) | 3.8 – 8.0 | **0.26 (0.06)** | **0.45 (0.04)** | 1.37 (0.06) | **0.84 (0.23)** | **0.41 (0.16)** | 1532 |
| KLH-IgG | 8.0 (1.6) | 5.1 – 10.3 | **0.16 (0.07)** | **0.45 (0.04)** | 2.10 (0.08) | **0.94 (0.24)** | **0.50 (0.18)** | 1519 |
| KLH-IgM | 7.0 (1.3) | 5.0 – 8.7 | **0.24 (0.07)** | **0.40 (0.05)** | 1.40 (0.06) | **0.78 (0.23)** | **0.47 (0.16)** | 1532 |
| MDP-IgA | 6.4 (1.4) | 4.0 – 8.6 | **0.29 (0.07)** | **0.38 (0.05)** | 1.61 (0.07) | **0.71 (0.26)** | **0.43 (0.17)** | 1532 |
| MDP-IgG | 8.4 (1.8) | 5.5 – 11.3 | **0.24 (0.07)** | **0.55 (0.04)** | 2.84 (0.12) | **0.89 (0.33)** | **0.61 (0.22)** | 1532 |
| MDP-IgM | 9.1 (1.4) | 6.5 – 10.9 | **0.22 (0.06)** | **0.37 (0.05)** | 1.78 (0.07) | **0.80 (0.25)** | **0.60 (0.17)** | 1532 |
| *First test milk* | | | | | | | | |
| KLH-IgA | 2.1 (1.4) | 1.2 – 5.2 | 0.03 (0.17) | - | 1.53 (0.14) | **0.46 (0.18)** | - | 272 |
| KLH-IgG | 3.1 (1.0) | 1.6 – 4.9 | **0.33 (0.17)** | - | 0.95 (0.09) | **0.48 (0.26)** | - | 272 |
| KLH-IgM | 2.4 (0.9) | 1.3 – 3.8 | 0.00 (0.00) | - | 0.72 (0.06) | **0.36 (0.11)** | - | 272 |
| MDP-IgA | 2.9 (1.3) | 1.3 – 5.4 | 0.15 (0.20) | - | 1.43 (0.14) | **0.45 (0.25)** | - | 248 |
| MDP-IgG | 2.9 (0.8) | 1.7 – 4.4 | 0.00 (0.00) | - | 0.66 (0.06) | **0.23 (0.11)** | - | 262 |
| MDP-IgM | 3.4 (1.2) | 1.8 – 5.7 | 0.03 (0.15) | - | 0.89 (0.08) | 0.18 (0.14) | - | 268 |
| *Pre-Parturition serum* | | | | | | | | |
| KLH-IgA | 2.6 (0.7) | 1.7 – 3.7 | **0.64 (0.16)** | - | 0.36 (0.03) | 0.29 (0.20) | - | 321 |
| KLH-IgG | 4.8 (1.3) | 2.7 – 7.2 | **0.52 (0.16)** | - | 1.38 (0.12) | **0.83 (0.36)** | - | 321 |
| KLH-IgM | 7.0 (1.1) | 5.4 – 8.9 | **0.37 (0.17)** | - | 1.14 (0.10) | 0.15 (0.29) | - | 321 |
| MDP-IgA | 3.3 (1.0) | 1.6 – 4.8 | **0.52 (0.15)** | - | 0.90 (0.08) | **0.48 (0.29)** | - | 320 |
| MDP-IgG | 3.1 (1.1) | 1.5 – 5.0 | 0.25 (0.14) | - | 0.93 (0.08) | **0.44 (0.22)** | - | 321 |
| MDP-IgM | 3.4 (0.9) | 2.0 – 4.9 | **0.40 (0.16)** | - | 0.66 (0.06) | 0.07 (0.22) | - | 321 |

[1] Significant $h^2$, $r$ and breed effects are highlighted in bold

[2] Range shows 5th quantile and 95th quantile, respectively

[3] Adjusted breed effects are compared to Swedish Red (SRB)

[4] Variance components and effects were estimated using a square root transformation
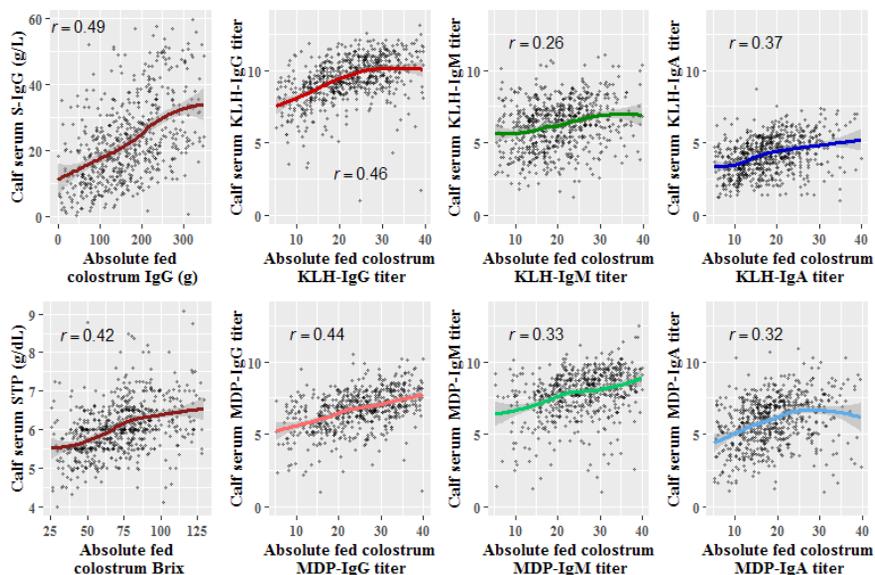
*Figure 2.* Scatterplots of calf serum traits vs. their matching absolute fed colostrum traits with a LOESS curve and Pearson correlation (r) value

Genetic correlations for colostrum traits are presented in Table 4. All significant genetic correlations were positive and ranged between 0.49 and 0.97. Most of them were among NAbs of the same isotype or between IgA and IgM. Brix was positively genetically correlated with all the traits, including total IgG.
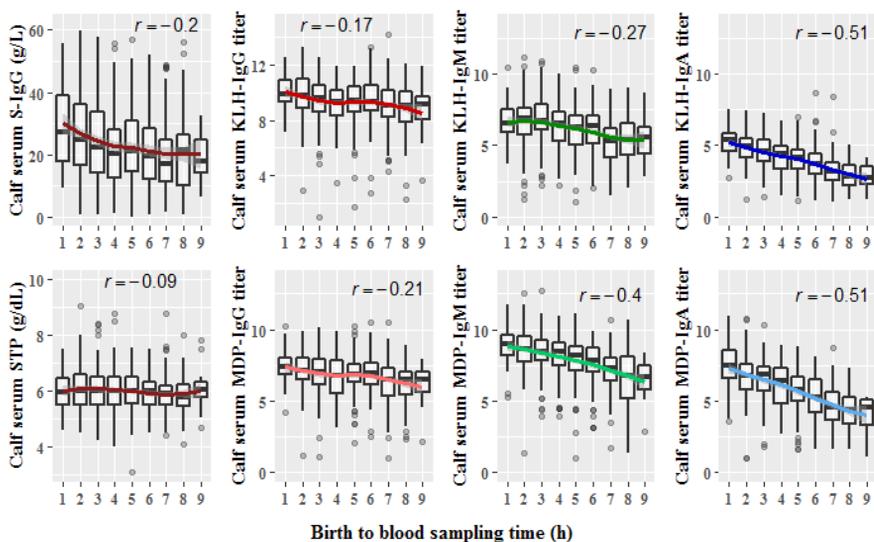


*Figure 3.* Plots of calf serum traits vs birth to blood sampling time with a LOESS curve and Pearson correlation (r) value

Table 4. *Estimated genetic correlations (below diagonal) and phenotypic correlations (above diagonal) between colostrum traits. SE in parenthesis*

| Trait[1] | Brix | T-IgG[2] | KLH-IgA | KLH-IgG | KLH-IgM | MDP-IgA | MDP-IgG | MDP-IgM |
|---|---|---|---|---|---|---|---|---|
| Brix | - | 0.70 (0.02) | 0.52 (0.02) | 0.51 (0.02) | 0.60 (0.02) | 0.48 (0.02) | 0.45 (0.02) | 0.58 (0.02) |
| T-IgG[2] | **0.68 (0.14)** | - | 0.37 (0.04) | 0.51 (0.03) | 0.48 (0.03) | 0.33 (0.04) | 0.35 (0.04) | 0.39 (0.03) |
| KLH-IgA | **0.71 (0.10)** | 0.23 (0.26) | - | 0.55 (0.02) | 0.78 (0.01) | 0.68 (0.01) | 0.45 (0.02) | 0.67 (0.02) |
| KLH-IgG | **0.66 (0.14)** | 0.93 (0.23)[3] | 0.39 (0.21)[3] | - | 0.57 (0.02) | 0.40 (0.02) | 0.56 (0.02) | 0.49 (0.02) |
| KLH-IgM | **0.73 (0.09)** | 0.22 (0.25) | **0.85 (0.06)** | 0.32 (0.23) | - | 0.67 (0.02) | 0.49 (0.02) | 0.79 (0.01) |
| MDP-IgA | **0.52 (0.13)** | 0.05 (0.28) | **0.92 (0.06)** | 0.13 (0.24) | **0.81 (0.09)** | - | 0.41 (0.02) | 0.67 (0.02) |
| MDP-IgG | **0.49 (0.14)** | 0.33 (0.26) | **0.56 (0.15)** | **0.76 (0.15)** | 0.37 (0.18) | 0.44 (0.17)[3] | - | 0.53 (0.02) |
| MDP-IgM | **0.68 (0.10)** | 0.13 (0.27) | **0.82 (0.08)** | 0.10 (0.29) | **0.97 (0.04)** | **0.92 (0.08)** | 0.34 (0.19) | - |

[1.] Significant genetic correlations are highlighted in bold
[2.] Variance components for correlations were estimated using a square root transformation
[3.] Borderline significant (0.05< *p-value* <0.10)

Something similar can be observed for genetic correlations among calf traits (Table 5.). Significant genetic correlations ranged from 0.62 to 0.96 and were mostly MDP-IgA and MDP-IgM with other traits, including S-IgG (0.87 and 0.81, respectively).

Phenotypic and genetic correlations were also estimated between colostrum and calf traits (Table 6.). There were no significant genetic correlations between STP and S-IgG with Brix or colostrum total IgG. Their phenotypic correlations, however, ranged from 0.17 to 0.26. Only IgA and IgM NAbs had significant genetic correlations, which ranged from 0.66 to 0.99.

Table 5. *Estimated genetic correlations (below diagonal) and phenotypic correlations (above diagonal) between calf serum traits. SE in parenthesis*

| Trait[1] | STP | S-IgG[2] | KLH-IgA | KLH-IgG | KLH-IgM | MDP-IgA | MDP-IgG | MDP-IgM |
|---|---|---|---|---|---|---|---|---|
| STP | - | 0.67 (0.02) | 0.49 (0.03) | 0.45 (0.04) | 0.37 (0.04) | 0.48 (0.03) | 0.43 (0.04) | 0.53 (0.03) |
| S-IgG[2] | 0.91 (0.18) | - | 0.57 (0.03) | 0.59 (0.03) | 0.40 (0.04) | 0.60 (0.03) | 0.62 (0.03) | 0.61 (0.03) |
| KLH-IgA | 0.61 (0.25) | 0.70 (0.16)[3] | - | 0.44 (0.04) | 0.53 (0.03) | 0.76 (0.02) | 0.49 (0.03) | 0.71 (0.02) |
| KLH-IgG | 0.71 (0.46) | 0.74 (0.20) | 0.23 (0.25) | - | 0.40 (0.04) | 0.53 (0.03) | 0.66 (0.03) | 0.54 (0.03) |
| KLH-IgM | 0.47 (0.52) | 0.59 (0.28) | 0.80 (0.14)[3] | 0.61 (0.28) | - | 0.51 (0.03) | 0.39 (0.04) | 0.62 (0.03) |
| MDP-IgA | 0.67 (0.31) | **0.87 (0.13)** | **0.91 (0.08)** | 0.86 (0.14)[3] | 0.90 (0.17)[3] | - | 0.59 (0.03) | 0.77 (0.02) |
| MDP-IgG | 0.83 (0.35) | 0.90 (0.15)[3] | 0.80 (0.16)[3] | 0.87 (0.13)[3] | 0.77 (0.25) | 0.99 (0.11) | - | 0.49 (0.03) |
| MDP-IgM | 0.83 (0.26) | **0.81 (0.12)** | **0.84 (0.09)** | **0.62 (0.18)** | **0.91 (0.11)** | **0.96 (0.06)** | 0.77 (0.02)[3] | - |

[1.] Significant genetic correlations are highlighted in bold
[2.] Variance components for correlations were estimated using a square root transformation
[3.] Borderline significant (0.05< *p-value* <0.10)

Table 6. *Genetic and phenotypic correlations between colostrum traits (vertical) and calf serum traits (horizontal). SE in parenthesis*

| Trait[1] | STP | S-IgG[2] | KLH-IgA | KLH-IgG | KLH-IgM | MDP-IgA | MDP-IgG | MDP-IgM |
|---|---|---|---|---|---|---|---|---|
| *Phenotypic correlations* | | | | | | | | |
| Brix | 0.17 (0.04) | 0.20 (0.04) | 0.14 (0.05) | 0.14 (0.05) | 0.10 (0.05) | 0.14 (0.05) | 0.10 (0.05) | 0.21 (0.04) |
| T-IgG[2] | 0.26 (0.04) | 0.26 (0.04) | 0.13 (0.04) | 0.13 (0.04) | 0.07 (0.04) | 0.13 (0.05) | 0.14 (0.04) | 0.17 (0.04) |
| KLH-IgA | 0.08 (0.04) | 0.13 (0.04) | 0.39 (0.04) | 0.21 (0.04) | 0.26 (0.04) | 0.32 (0.04) | 0.20 (0.04) | 0.33 (0.04) |
| KLH-IgG | 0.09 (0.05) | 0.17 (0.04) | 0.15 (0.04) | 0.53 (0.03) | 0.09 (0.05) | 0.09 (0.05) | 0.28 (0.04) | 0.14 (0.05) |
| KLH-IgM | 0.12 (0.04) | 0.16 (0.04) | 0.30 (0.04) | 0.14 (0.04) | 0.25 (0.04) | 0.34 (0.04) | 0.16 (0.04) | 0.40 (0.04) |
| MDP-IgA | 0.08 (0.05) | 0.12 (0.05) | 0.27 (0.04) | 0.14 (0.04) | 0.19 (0.04) | 0.35 (0.04) | 0.19 (0.04) | 0.31 (0.04) |
| MDP-IgG | 0.04 (0.04) | 0.07 (0.04) | 0.13 (0.04) | 0.13 (0.04) | 0.04 (0.04) | 0.06 (0.05) | 0.43 (0.04) | 0.11 (0.04) |
| MDP-IgM | 0.13 (0.04) | 0.13 (0.04) | 0.26 (0.04) | 0.14 (0.04) | 0.25 (0.04) | 0.29 (0.04) | 0.12 (0.04) | 0.37 (0.04) |
| *Genetic correlations* | | | | | | | | |
| Brix | 0.54 (0.38) | 0.61 (0.54) | 0.39 (0.22) | 0.70 (0.61) | -0.02 (0.32) | 0.31 (0.22) | N.E.[4] | 0.40 (0.19) |
| T-IgG[2] | 0.95 (0.32) | 0.82 (0.52) | 0.43 (0.28) | 0.86 (0.63) | -0.08 (0.40) | 0.18 (0.30) | 0.88 (0.66) | 0.48 (0.23) |
| KLH-IgA | 0.58 (0.44) | 0.51 (0.69) | **0.94 (0.18)** | 0.23 (0.84) | 0.70 (0.27)[3] | **0.71 (0.19)** | 0.85 (0.50) | **0.78 (0.15)** |
| KLH-IgG | 0.60 (0.42) | 0.77 (0.59) | 0.11 (0.40) | 0.99 (0.42) | -0.51 (0.54) | -0.31 (1.61) | 0.93 (0.38) | -0.02 (0.32) |
| KLH-IgM | 0.75 (0.52) | 0.95 (0.89) | **0.98 (0.18)** | 0.34 (0.74) | 0.67 (0.28)[3] | **0.67 (0.18)** | 0.84 (0.59) | **0.91 (0.13)** |
| MDP-IgA | 0.52 (0.52) | 0.68 (0.96) | **0.99 (0.22)** | 0.36 (0.61) | 0.48 (0.30) | **0.65 (0.17)** | 0.90 (0.65) | **0.77 (0.17)** |
| MDP-IgG | 0.30 (0.44) | 0.18 (0.65) | 0.51 (0.31) | -0.16 (0.76) | 0.03 (0.34) | 0.29 (0.34) | 0.91 (0.45) | 0.13 (0.26) |
| MDP-IgM | 0.99 (0.72) | 0.92 (1.06) | **0.98 (0.18)** | 0.43 (0.64) | **0.89 (0.27)** | **0.96 (0.29)** | 0.60 (0.62) | **0.99 (0.23)** |

[1.] Significant genetic correlations are highlighted in bold

[2.] Variance components for correlations were estimated using a square root transformation

[3.] Borderline significant ($0.05 < p\text{-value} < 0.10$)

[4.] Not estimable

Genetic correlations of colostrum traits with NAbs of first test milk are shown in Table 7. Only colostrum NAbs had significant genetic correlations with milk NAbs, and ranged from 0.80 to 0.97, mostly from colostrum MDP-IgG with milk NAbs. Genetic correlations were also estimated for pre-parturition serum, having no significant correlations with Brix or total IgG, while NAb correlations were between 0.63 and 0.98. Genetic correlations of milk production traits for 305d including milk yield (kg), LASCS, fat percentage and protein percentage with colostrum traits were estimated, however, none of the traits were significantly correlated.

Table 7. *Genetic correlations of colostrum traits (horizontal) versus first test milk, pre-parturition serum traits and milk production traits. SE in parenthesis*

| Trait[1] | Brix | T-IgG[2] | KLH-IgA | KLH-IgG | KLH-IgM | MDP-IgA | MDP-IgG | MDP-IgM |
|---|---|---|---|---|---|---|---|---|
| *First test milk* | | | | | | | | |
| KLH-IgA | 0.14 (0.39) | 0.43 (0.55) | -0.54 (0.51) | 0.93 (0.51) | -0.45 (0.48) | -0.21 (0.41) | **0.86 (0.46)** | -0.07 (0.44) |
| KLH-IgG | 0.66 (0.37) | 0.26 (0.43) | 0.47 (0.36) | 0.79 (0.36) | 0.58 (0.33) | 0.41 (0.35) | **0.98 (0.37)** | 0.43 (0.34) |
| KLH-IgM | 0.83 (0.84) | 0.68 (0.89) | 0.99 (0.75) | 0.78 (0.40) | **0.87 (0.20)** | **0.84 (0.24)** | **0.80 (0.30)** | **0.97 (0.21)** |
| MDP-IgA | 0.02 (0.37) | -0.22 (0.53) | 0.59 (0.35) | 0.82 (0.71) | 0.50 (0.43) | 0.59 (0.56) | 1.00 (0.70) | 0.47 (0.45) |
| MDP-IgG | 0.86 (0.64) | 0.61 (1.25) | 0.83 (0.49) | 0.83 (0.64) | 0.75 (0.28)[3] | 0.59 (0.33) | **0.97 (0.30)** | 0.59 (0.67) |
| MDP-IgM | -0.25 (4.68) | -0.24 (4.79) | 1.00 (0.99) | N.E.[4] | **0.98 (0.29)** | 0.72 (0.44) | N.E.[4] | 0.92 (1.40) |
| *Pre-parturition serum* | | | | | | | | |
| KLH-IgA | -0.21 (0.23) | -0.13 (0.30) | 0.40 (0.23) | 0.20 (0.32) | **0.63 (0.23)** | **0.92 (0.17)** | 0.55 (0.26)[3] | **0.80 (0.19)** |
| KLH-IgG | -0.12 (0.23) | 0.30 (0.31) | -0.02 (0.25) | 0.60 (0.36) | -0.40 (0.29) | -0.22 (0.26) | 0.43 (0.26) | -0.44 (0.26) |
| KLH-IgM | -0.19 (0.32) | -0.59 (0.42) | 0.48 (0.36) | 0.45 (0.49) | 0.58 (0.38) | 0.94 (0.52)[3] | 0.45 (0.39) | **0.85 (0.33)** |
| MDP-IgA | -0.28 (0.23) | 0.03 (0.31) | 0.45 (0.24) | 0.23 (0.33) | 0.39 (0.25) | **0.79 (0.21)** | **0.98 (0.24)** | **0.68 (0.24)** |
| MDP-IgG | 0.04 (0.33) | 0.35 (0.43) | **0.70 (0.32)** | **0.99 (0.35)** | -0.03 (0.35) | 0.41 (0.31) | **0.82 (0.35)** | -0.16 (0.37) |
| MDP-IgM | -0.43 (0.29) | -0.30 (0.35) | 0.43 (0.30) | -0.04 (0.44) | 0.36 (0.31) | **0.89 (0.30)** | 0.23 (0.33) | **0.79 (0.31)** |
| *Milk production* | | | | | | | | |
| Milk yield | 0.25 (0.17) | -0.02 (0.28) | 0.04 (0.18) | 0.36 (0.26) | -0.07 (0.19) | 0.12 (0.18) | 0.15 (0.20) | -0.10 (0.20) |
| LASCS | N.E.[4] | 0.03 (0.29) | N.E.[4] | N.E.[4] | N.E.[4] | -0.42 (0.87) | 0.21 (0.27) | -0.15 (0.24) |
| Fat % | -0.15 (0.13) | 0.15 (0.21) | N.E.[4] | N.E.[4] | N.E.[4] | 0.00 (0.14) | 0.09 (0.15) | 0.08 (0.15) |
| Protein % | 0.00 (0.12) | 0.23 (0.20) | N.E.[4] | N.E.[4] | N.E.[4] | 0.08 (0.13) | 0.14 (0.14) | 0.13 (0.14) |

[1.] Significant genetic correlations are highlighted in bold

[2.] Variance components for correlations were estimated using a square root transformation

[3.] Borderline significant ($0.05 < p\text{-}value < 0.10$)

[4.] Not estimable

Phenotypic and genetic correlations of calf serum with calf health and production traits later in life are presented in Table 8. Only LASCS had significant correlations with calf serum, ranging from −0.66 to −0.98 for IgM NAb and MDP-IgA. Calf health had no significant genetic correlations, but it had a borderline significant ($p = 0.08$) negative genetic correlation with MDP-IgG and a consistent negative tendency can be seen on calf health versus all the other traits, both on phenotypic and genetic correlations.

Table 8. *Genetic and phenotypic correlations of calf serum traits versus milk production and health traits*

| Trait[1,2] | STP | S-IgG[3] | KLH-IgA | KLH-IgG | KLH-IgM | MDP-IgA | MDP-IgG | MDP-IgM |
|---|---|---|---|---|---|---|---|---|
| *Phenotypic correlations* | | | | | | | | |
| Calf health | -0.04 (0.06) | -0.11 (0.07) | -0.08 (0.06) | -0.05 (0.06) | -0.12 (0.07) | -0.12 (0.06) | -0.12 (0.06) | -0.12 (0.07) |
| Adult health | -0.04 (0.05) | -0.06 (0.05) | 0.00 (0.05) | -0.04 (0.05) | 0.04 (0.05) | -0.08 (0.05) | -0.02 (0.05) | 0.03 (0.05) |
| AFC | -0.04 (0.08) | 0.03 (0.08) | -0.13 (0.08) | -0.16 (0.08) | 0.03 (0.08) | 0.05 (0.08) | -0.01 (0.08) | -0.11 (0.08) |
| Fat % | -0.12 (0.07) | -0.02 (0.07) | -0.02 (0.07) | -0.09 (0.07) | -0.03 (0.07) | -0.08 (0.07) | -0.04 (0.07) | -0.05 (0.07) |
| Milk yield | -0.03 (0.07) | -0.06 (0.08) | -0.04 (0.07) | -0.07 (0.07) | -0.09 (0.08) | -0.11 (0.07) | -0.05 (0.08) | -0.11 (0.08) |
| Protein % | 0.02 (0.07) | 0.06 (0.07) | 0.02 (0.07) | -0.01 (0.07) | 0.06 (0.07) | 0.05 (0.07) | 0.05 (0.07) | 0.02 (0.07) |
| LASCS | -0.09 (0.07) | -0.12 (0.07) | 0.01 (0.07) | 0.05 (0.07) | -0.12 (0.07) | -0.19 (0.07) | -0.10 (0.07) | 0.07 (0.07) |
| *Genetic correlations* | | | | | | | | |
| Calf health | 0.01 (0.75) | -0.42 (0.58) | -0.52 (0.47) | -0.72 (0.81) | -0.74 (0.58) | -0.53 (0.41) | -0.93 (0.41)[4] | -0.77 (0.47) |
| Adult health | 1.00 (1.43) | 0.36 (0.63) | 0.87 (1.05) | 0.14 (0.61) | 0.74 (0.76) | 0.44 (0.59) | 0.68 (0.89) | 0.08 (0.35) |
| AFC | -0.18 (0.25) | 0.09 (0.18) | -0.27 (0.16) | -0.44 (0.18)[4] | -0.06 (0.19) | -0.02 (0.14) | -0.09 (0.16) | -0.23 (0.16) |
| Fat % | -0.18 (0.22) | 0.00 (0.18) | -0.14 (0.16) | -0.08 (0.17) | 0.06 (0.19) | -0.13 (0.14) | -0.16 (0.17) | -0.11 (0.15) |
| Milk yield | -0.38 (0.27) | -0.20 (0.20) | 0.00 (0.18) | 0.06 (0.20) | -0.20 (0.21) | -0.32 (0.16) | -0.16 (0.19) | -0.28 (0.17) |
| Protein % | 0.21 (0.21) | 0.21 (0.17) | -0.06 (0.16) | -0.16 (0.18) | 0.06 (0.18) | 0.10 (0.14) | 0.18 (0.16) | 0.12 (0.15) |
| LASCS | -0.52 (0.33) | -0.62 (0.26)[4] | -0.38 (0.25) | -0.63 (0.26)[4] | **-0.98 (0.26)** | **-0.66 (0.22)** | -0.74 (0.30)[4] | **-0.86 (0.23)** |

[1.] Significant genetic correlations are highlighted in bold

[2.] Calf health refers to the Healthy/Disease data for calves between 0 to 3 months of age, Adult health refers to the Healthy/Disease data for animals between 8 to 36 months of age

[3.] Variance components for correlations were estimated using a square root transformation

[4.] Borderline significant (0.05< *p-value* <0.10)

## 4.2 Total IgG and natural antibodies in colostrum of Swedish dairy cows: a genome-wide association study (Paper II)

In the present study we analyzed eight colostrum traits comprising Brix, total IgG, and NAb isotypes IgA and IgM titers binding KLH and MDP. The GWAS was performed based on 829 animals, which had information for colostrum phenotypes and 50K imputed genotypes. Out of 45,823 SNPs, a total of 39,990 were used for the analysis after quality control. Inflation factor λ ranged between 0.95 and 1.13, so no genomic-control correction was applied.

Table 9. *Genomic regions and lead SNPs suggestive or significantly associated with colostrum traits*

| Chromosome | Position range (bp) | Trait | SNP and Position | MAF[1] | $-\log_{10}$ *p-value* |
|---|---|---|---|---|---|
| 3 | 39,399,136 – 43,134,780 | MDP-IgM | rs29001941 40,399,136 | 0.46 | 4.2[2] |
| | | Brix | rs29018907 42,134,780 | 0.39 | 4.6[2] |
| 7 | 73,537,346 – 75,537,346 | KLH-IgM MDP-IgA | rs42376828 74,537,346 | 0.41 | 4.8[2] |
| 9 | 35,790,663 – 37,790,663 | T-IgG | rs110760584 36,790,663 | 0.20 | 4.5[2] |
| 11 | 11,088,913 – 13,088,913 | MDP-IgA | rs41811845 12,088,913 | 0.26 | 4.9[2] |
| | 42,886,198 – 49,155,240 | T-IgG | rs41257374 43,886,198 | 0.25 | 6.1 |
| 15 | 23,112,375 – 31,840,190 | KLH-IgM MDP-IgM | rs717997787 23,112,375 | 0.31 | 5.5[2] 4.5[2] |
| 20 | 5,310,930 – 7,310,930 | T-IgG | rs109309318 6,310,930 | 0.33 | 4.7[2] |
| | 50,511,878 – 53,398,914 | T-IgG MDP-IgG | rs109317358 52,398,914 | 0.24 | 6.1 4.4[2] |
| 24 | 42,665,926 – 43,762,598 | KLH-IgM MDP-IgM | rs110959252 42,665,926 | 0.22 | 4.9[2] |

[1.] Minor Allele Frequency

[2.] Suggestive association (0.05<FDR<0.20)

Traits that had some significant or suggestive associations were: Brix, T-IgG, KLH-IgM, MDP-IgG, MDP-IgM and MDP-IgA (Figure 4). No genomic regions

were found significantly nor suggestively associated with KLH-IgA or KLH-IgG. Lead SNPs for genomic regions with significant or suggestive associations are presented in Table 9.

*IgG phenotypes*

Two significant regions were found for total IgG. The strongest association was on BTA20, including one significant and three suggestive SNPs, MDP-IgG had one suggestive SNP in this region, which was also the lead SNP for total IgG; HAPMAP26426-BTA-150179 (rs109317358) at 52,398,914 bp. The other region was on BTA11 with one significant SNP; HAPMAP58586-SS46526926 (rs41257374) at 43,886,198 bp.

Two more SNP on different chromosomes were suggestively associated with total IgG. BTA9 with BTA-21753-NO-RS (rs110760584) at 36,790,663 bp and BTA20 with BFGL-NGS-118422 (rs109309318) at 6,310,930 bp.

*IgM and IgA phenotypes*

Five regions with suggestive associations were found for IgA and IgM traits. Most of these regions have only one or two SNP associations, but in most cases two different traits shared the same lead SNP. The strongest association was found on BTA15 for KLH-IgM and MDP-IgM comprised of two suggestive SNPs, with HAPMAP42921-BTA-36160 (rs717997787) at 23,112,375 bp as the lead SNP. Another region was found for KLH-IgM and MDP-IgM on BTA24 with two suggestive SNPs and ARS-BFGL-NGS-95379 (rs110959252) at 42,665,926 bp as lead SNP.

BTA11 had one SNP associated with MDP-IgA; BTB-00649769 (rs41811845) at 12,088,913 bp. BTA7 also had only one suggestive SNP, but found for both KLH-IgM and MDP-IgA the SNP was BTB-01217732 (rs42376828) at 74,537,346 bp. One more region was found on BTA3 for Brix and MDP-IgM, in both cases only one SNP was significant, but it was not the same.

*Candidate genes*

An overview of candidate genes and selected pathways for significant and suggestive genomic regions is presented in Table 10. Genes associated with immunity functions were selected, prioritizing genes close to the lead SNP in said region.

Table 10. *Candidate genes and selected pathways in suggestive and significant genomic regions for colostrum traits*

| Chromosome | Region (Mbp) | Trait | Gene symbol | Description | Pathway (source) |
|---|---|---|---|---|---|
| 3 | 39.4 – 43.1 | MDP-IgM Brix | *VCAM1* | Vascular cell adhesion molecule 1 | Leukocyte transendothelial migration, TNF signaling, NF-kappa B signaling (KEGG) |
| | | | *LOC534578* | Vascular cell adhesion molecule 1-like | Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell, Integrin cell surface interactions (Reactome) |
| 7 | 73.5 – 75.5 | KLH-IgM MDP-IgA | *IL12B* | Interleukin 12B | Interleukin-12 family signaling (Reactome) Cytokines and Inflammatory Response, Toll-like receptor signaling (Wikipathway) |
| 9 | 35.8 – 37.8 | T-IgG | *FYN* | FYN proto-oncogene, Src family tyrosine kinase | Interleukin 2, 3, 11 and 17 signaling, Interferon type I signaling, T cell receptor and co-stimulatory signaling, Focal adhesion (Wikipathway) Integrin signaling (Panther) |
| | | | *TRAF3IP2* | TRAF3 interacting protein 2 | Positive regulation of I-kappaB kinase/NF-kappaB signaling, B cell apoptotic process, Immunoglobulin secretion (Panther) |
| 11 | 42.8 – 49.1 | T-IgG | *CD8A, CD8B, NCK2* | CD8a molecule, CD8b molecule, NCK adaptor protein 2 | T cell receptor signaling, Primary immunodeficiency (KEGG) T cell receptor and co-stimulatory signaling (Wikipathway) |
| | | | *IL1, IL36, IL37* | Interleukin 1, 36 and 37 | Cytokine-cytokine receptor interaction (KEGG) |
| 15 | 23.1 – 31.8 | KLH-IgM MDP-IgM | *CD3D, CD3E, CD3G* | CD3d molecule, CD3e molecule, CD3g molecule | Th1 and Th2 cell differentiation, T cell receptor signaling, Primary immunodeficiency (KEGG) |
| 20 | 50.5 – 53.4 | T-IgG MDP-IgG | *MYO10* | Myosin X | Fc gamma receptor (FCGR) mediated phagocytosis (KEGG) |

BTA20 had the strongest association in the GWAS with total IgG and MDP-IgG. We propose Myosin X (*MYO10*) as a candidate gene, which according to the KEGG database is involved in Fc gamma receptor (*FCGR*) mediated phagocytosis. The second strongest association was on BTA11 for total IgG as well and we propose six candidate genes for this region: CD8a and b molecules (*CD8A* and *CD8B*, respectively) and NCK adaptor protein 2 (*NCK2*), related to T cell receptor and co-stimulatory signaling according to KEGG and Wikipathway databases and Interleukins 1, 36 and 37 (*IL1*, *IL36* and *IL37*, respectively) related to immune response regulation.

For BTA9 two candidate genes are proposed: FYN, a proto-oncogene with a broad role, including T cell regulation and TRAF3 interacting protein 2 (*TRAF3IP2*), a molecule that binds TRAF3, has been shown to interact with inhibitor of nuclear factor kappa-B kinase subunit gamma (IKK-γ) and is involved in B cell apoptotic process and immunoglobulin secretion according to Panther database.
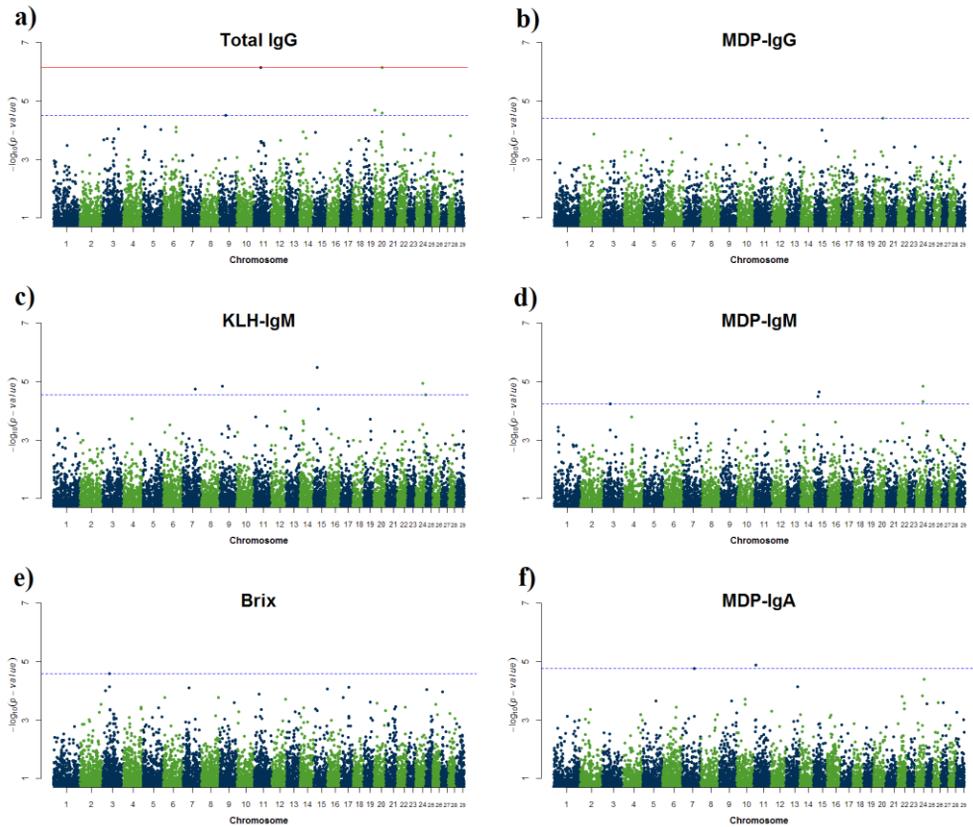


*Figure 4.* GWAS plots on colostrum traits **a)** Total-IgG **b)** MDP-IgG **c)** KLH-IgM **d)** MDP-IgM **e)** Brix **f)** MDP-IgA. The y-axis represents the −log10(*p-value*) of the SNP association with the trait. The false discovery rate (FDR) threshold was set at 0.05 for significant SNP (solid line) and at 0.20 for suggestive SNP (dotted line)

In the case of BTA15, three candidate genes were found: CD3d, e and g molecules (*CD3D*, *CD3E* and *CD3G*, respectively) involved in Th1 and Th2 cell differentiation and T cell receptor signaling (KEGG database). One candidate gene was found for BTA 7: Interleukin 12B (*IL12B*) related to inflammatory response and Toll-like receptor signaling (Wikipathways).

Two candidate genes were proposed for BTA3, which had associations for Brix and MDP-IgM. One is vascular cell adhesion molecule 1 (*VCAM1*) and the other one vascular cell adhesion molecule 1-like (LOC534578), which according to Reactome database are related to leukocyte transendothelial migration, TNF signaling, NF-kappa B signaling and immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell.

## 4.3 Genomic regions associated with antibody uptake in newborn calves (Paper III)

In this paper we analyzed eight calf serum traits including STP, S-IgG, and NAb isotypes IgG, IgA and IgM titers binding KLH and MDP. The GWAS was performed based on 708 animals, which had information for calf serum phenotypes and 50K imputed genotypes. Out of 45,823 SNPs, a total of 39,029 were used for the analysis after quality control. Inflation factor λ ranged between 1.04 and 1.1, so no genomic-control correction was applied.

We found five genomic regions significantly associated with calf serum traits on five different chromosomes. Additionally, nine suggestive regions were found on eight other chromosomes. Traits with significant and/or suggestive associations were: S-IgG, KLH-IgG, MDP-IgG and KLH-IgM (Figure 5). No genomic regions were found significantly or suggestively associated with any of the IgA traits, STP or MDP-IgM. Lead SNPs for genomic regions with significant or suggestive associations are presented in Table 11.

*IgG phenotypes*

All five significantly associated regions were found on IgG traits. BTA7 had the strongest association, but just one SNP for KLH-IgG; ARS-BFGL-NGS-64882 (rs110105248) at 82,923,398 bp with a $-\log10(p\text{-}value)$ of 6.6. The next region had two SNPs, located on BTA15 with one significant and one suggestive SNP and ARS-BFGL-NGS-21421 (rs41762578) at 35,298,693 bp as lead SNP. Next, BTA6 had only one significant SNP, BTA-76960-NO-RS (rs41654429) at 83,229,855 bp for two traits; S-IgG and MDP-IgG. BTA1 had one significant and one suggestive SNP for MDP-IgG, having BTA-123503-NO-RS (rs43212241) at 5,351,369 bp as lead SNP. Finally, MDP-IgG had one significant SNP on BTA4; ARS-BFGL-NGS-74447 (rs109798792) at 119,753,498 bp.

Five additional suggestive associations were found for IgG. BTA3 had one SNP, BTB-01898997 (rs43005354) at 46,977,886 bp for S-IgG. On BTA10, two suggestive SNPs for MDP-IgG were found, with ARS-BFGL-NGS-66047 (rs110844478) at 66,174,661 bp. Next, BTA1 had one suggestive association for S-IgG at 126,567,481 bp with SNP ARS-BFGL-NGS-98257 (rs43272464). One suggestive SNP (HAPMAP54258 (rs29018641) was found on BTA20 for KLH-IgG at 31,151,035 bp. Lastly, on BTA27 one suggestive SNP was found for KLH-IgG (ARS-BFGL-NGS-109626 (rs110979317) at 17,846,556 bp.

Table 11. *Genomic regions and lead SNPs suggestive or significantly associated with calf serum traits*

| Chromosome | Position range (bp) | Trait | SNP and Position | MAF[1] | $-\log_{10}$ *p-value* |
|---|---|---|---|---|---|
| 1 | 4,351,369 – 6,374,084 | MDP-IgG | rs43212241 5,351,369 | 0.19 | 5.7 |
| | 125,567,481 – 127,567,481 | S-IgG | rs43272464 126,567,481 | 0.43 | 4.6[2] |
| 3 | 45,977,886 – 47,977,886 | S-IgG | rs43005354 46,977,886 | 0.34 | 4.9[2] |
| 4 | 70,484,912 – 79,876,832 | KLH-IgM | rs109428435 78,876,832 | 0.44 | 4.6[2] |
| | 118,753,498 – 120,753,498 | MDP-IgG | rs109798792 119,753,498 | 0.10 | 5.3 |
| 6 | 82,229,855 – 84,229,855 | S-IgG MDP-IgG | rs41654429 83,229,855 | 0.41 | 5.8 4.3[2] |
| | 88,104,201 – 90,251,522 | KLH-IgM | rs41663978 89,133,819 | 0.14 | 5.3[2] |
| 7 | 81,923,398 – 83,923,398 | KLH-IgG | rs110105248 82,923,398 | 0.50 | 6.6 |
| 10 | 65,090,075 – 67,174,661 | MDP-IgG | rs110844478 66,174,661 | 0.30 | 4.8[2] |
| 11 | 60,037,082 – 62,037,082 | KLH-IgM | rs41633408 61,037,082 | 0.26 | 4.3[2] |
| 15 | 34,252,207 – 36,298,693 | KLH-IgG | rs41762578 35,298,693 | 0.24 | 6.5 |
| 20 | 30,151,035 – 32,151,035 | KLH-IgG | rs29018641 31,151,035 | 0.40 | 4.2[2] |
| | 51,816,141 – 53,816,141 | KLH-IgM | rs110058467 52,816,141 | 0.25 | 4.5[2] |
| 27 | 16,846,556 – 18,846,556 | KLH-IgG | rs110979317 17,846,556 | 0.37 | 4.4[2] |

[1.] Minor Allele Frequency

[2.] Suggestive association (0.05<FDR<0.20)

*IgM phenotypes*

Four suggestive associations were found for IgM, all KLH-IgM. BTA6 had three suggestive SNPs and the strongest lead SNP ARS-BFGL-NGS-28041

(rs109201532) at 89,251,522 bp with a $-\log 10(p\text{-}value)$ of 5.3. BTA4 one suggestive association for SNP BFGL-NGS-112048 (rs109428435) at 78,876,832 bp. BTA20 also had one suggestive SNP ARS-BFGL-NGS-51112 (rs110058467) at 52,816,141 bp. Finally, BTA11 had one SNP HAPMAP39736-BTA-26188 (rs41633408) 61,037,082 bp.

Table 12. *Candidate genes and selected pathways in suggestive and significant genomic regions for calf serum traits*

| Chromosome | Region (Mbp) | Trait | Gene symbol | Description | Pathway (source) |
|---|---|---|---|---|---|
| 1 | 4.3 – 6.3 | MDP-IgG | *CLDN8, CLDN17* | Claudin 8 and 17 | Leukocyte transendothelial migration, Tight junction, Cell adhesion molecules (CAMs) (KEGG) |
| 4 | 70.5 – 79.9 | KLH-IgM | *ADCY1 CAMK2B* | Adenylate cyclase 1, Calcium/calmodulin dependent protein kinase II beta | Gastric acid secretion, Insulin secretion (KEGG) |
| | 118.7 – 120.7 | MDP-IgG | *VIPR2* | Vasoactive intestinal peptide receptor 2 | GPCRs, class B secretin-like (Wikipathway) |
| 6 | 82.3 – 84.2 | S-IgG MDP-IgG | *HSTN, MUC7, STATH* | Histatherin, Mucin 7, Statherin | Salivary secretion (KEGG) |
| | 88.1 – 90.2 | KLH-IgM | *GRO1, CXCL2, 3, 5, 8* | Chemokine (C-X-C motif) ligand 1, 2, 3, 5, 8 | Cytokine-cytokine receptor interaction, Chemokine signaling (KEGG) |
| 10 | 65.1 – 67.2 | MDP-IgG | *DUOX2* | Dual oxidase 2 | NOD pathway (Wikipathway) |
| 11 | 60.0 – 62.0 | KLH-IgM | *PELI1* | Pellino E3 ubiquitin protein ligase 1 | Interleukin 1 signaling, Regulation of toll-like receptor signaling (Wikipathway) IRAK1 recruits IKK complex (Reactome) |
| 20 | 51.8 – 53.8 | KLH-IgM | *MYO10* | Myosin X | Fc gamma receptor (FCGR) mediated phagocytosis (KEGG) |
| 27 | 16.8 – 18.8 | KLH-IgG | *TLR3* | Toll-like receptor 3 | Toll-like receptor signaling (KEGG) |
| | | | *LOC112444630, LOC536739* | Putative claudin 24, Claudin 22 | Leukocyte transendothelial migration, Tight junction, Cell adhesion molecules (CAMs) (KEGG) |

*Candidate genes*

Candidate genes within genomic regions significantly and suggestively associated with calf serum traits titers and pathways are presented in Table 12. Genes associated with immunity and molecule transport were selected, prioritizing genes close to the lead SNP in said region.

For BTA6 region 82.3-84.2 Mbp, we found three candidate genes: Histatherin (*HSTN*), Mucin 7 (*MUC7*) and Statherin (*STATH*) related to salivary secretion according to KEGG database. In the case of BTA1 REGION 4.4 to 6.4 Mbp, two candidate genes were identified: Claudin 8 (*CLDN8*) and Claudin 17 (*CLDN17*), which according to KEGG database, are related to Leukocyte transendothelial migration, Tight junction and Cell adhesion molecules (CAMs). On BTA4, region 119-121 Mbp we found Vasoactive intestinal peptide receptor 2 (*VIPR2*) gene, known to have various roles in immunity and it also is associated with water and ion regulation in the intestine.

In the case of BTA10 (65-67 Mbp) Dual oxidase 2 (*DUOX2*) is proposed as candidate gene part of the NOD pathway according to Wikipathway and it is present in the salivary glands and gastrointestinal tract. It is also known to be stimulated by MDP. For BTA27 17-19 Mbp region, three candidate genes were identified: Toll-like receptor 3 (*TLR3*), Putative claudin-24 (*LOC112444630*) and claudin-22 (*LOC536739*). TLR3 is known for its role in PAMP recognition (such as MDP) and activation of innate immunity. Putative claudin-24 and claudin-22 are predicted genes based on sequences associated to Leukocyte transendothelial migration and Tight junction according to KEGG database.

For BTA6 region 88-90 Mbp, five candidate genes were found: Chemokine (C-X-C motif) ligand 1, 2, 3, 5 and 8 (*GRO1*, *CXCL2*, *3*, *5* and *8*, respectively), which play different roles in the immune system. BTA4, region 71 to 80 Mbp, had two candidate genes: Adenylate cyclase 1 (*ADCY1*) and Calcium/calmodulin dependent protein kinase II beta (*CAMK2B*) related to gastric acid and insulin secretion (KEGG). On BTA20, region 52 to 54 Mbp, Myosin X (*MYO10*) is proposed as a candidate gene, which according to the KEGG database is involved in Fc gamma receptor (*FCGR*) mediated phagocytosis. In the case of BTA11 at 60-62 Mbp, one candidate gene is proposed: Pellino E3 ubiquitin protein ligase 1 (*PELI1*) related to Interleukin 1 signaling and Regulation of toll-like receptor signaling (Wikipathway).

BTA7 82-84 Mbp region had the strongest GWAS association with KLH-IgG as a trait, but no candidate genes could be identified, however, genes related to autophagy were found in this region. The same was true for region 125-127 Mbp on BTA1, associated with S-IgG, it was not possible to establish candidate genes, but the region has some genes related to carbohydrate digestion and absorption and endocrine-regulated calcium reabsorption. Candidate genes

could not be established either for BTA3 46-48 Mbp on S-IgG, but genes related to glycogen breakdown (glycogenolysis), SLC-mediated transmembrane transport and ATP binding cassette transporters were found in this region. For BTA 15 (KLH-IgG) no candidate genes were identified, but this region contains genes related to calcitonin-like ligand receptors and ATP-binding cassette (ABC) proteins mediated transport. In the case of BTA20, 30-32 Mbp region, no candidate genes nor related pathways could be found.
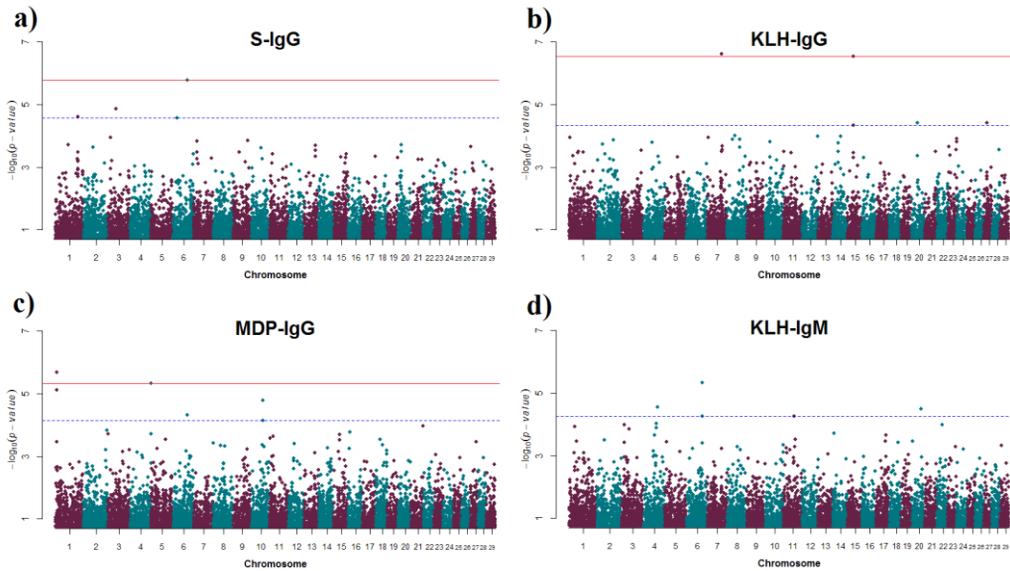


*Figure 5.* GWAS plots on calf serum traits **a)** Serum IgG **b)** KLH-IgG **c)** MDP-IgG **d)** KLH-IgM. The y-axis represents the $-\log10(p\text{-}value)$ of the SNP association with the trait. The false discovery rate (FDR) threshold was set at 0.05 for significant SNP (solid line) and at 0.20 for suggestive SNP (dotted line)

## 4.4 Genome-wide association study identifies loci influencing natural antibody titers in milk of Dutch Holstein-Friesian cattle (Paper IV)

In this paper, we analyzed 16 traits including total NAb titers binding KLH, LPS, LTA or PGN without isotype distinction, and NAb isotypes IgA, IgG1 and IgM titers binding KLH, LPS, LTA and PGN. The GWAS was performed based on 1,630 animals, that had information for NAbs phenotypes and 777K imputed genotypes. Out of 664,119 SNPs, a total of 567,461 were used for the analysis after quality control. Inflation factor λ ranged between 1.09 and 1.31, genomic-control correction was applied on all traits for consistency.

Traits with significant and/or suggestive associations were: LPS-IgG1, PGN-IgG1, LTA-IgG1, LTA-IgM and PGN-IgM (Figure 6). No genomic regions were found significantly nor suggestively associated with any of the KLH traits and neither IgA nor total NAbs for the other antigens. Lead SNPs for genomic regions with significant or suggestive associations are presented in Table 13.

*IgG1 phenotypes*

Two genomic regions were found significantly associated with IgG1 antibodies. The strongest one was on chromosome 21 (**BTA21**) for PGN-IgG1, spanning approximately from 65 M base pairs (**bp**) to 71 Mbp. It includes 36 significant and 55 suggestive SNPs, with the lead SNP, BovineHD2100020570 (rs137230097) located at 70,399,861 bp. The other region was on BTA3 for PGN-IgG1 and LPS-IgG1, around 6 to 8 Mbp. It consisted of 3 significant and 12 suggestive SNPs for PGN-IgG1, and 6 significant and 7 suggestive for LPS-IgG1. They did not share the lead SNP, with ARS-BFGL-NGS-25819 (rs110900415) at 7,416,886 bp for PGN-IgG1 and BovineHD0300002588 (rs109075424) at 7,931,301 bp for LPS-IgG1.

Six other regions were suggestively associated with IgG1 traits. However, no traits had any overlapping region. For PGN-IgG1, two regions were found; one in BTA10 and the other one in BTA24 with lead SNPs around 31.5 Mbp and 18.1 Mbp, respectively. LPS-IgG1 had three regions in two chromosomes with BTA2 having the lead SNP at 133.3 Mbp and BTA9 containing two regions with lead SNPs at 34.4 Mbp and 67.5 Mbp. LTA-IgG1 had only one region at BTA29 peaking at approximately 15.1 Mbp.

Table 13. *Genomic regions and lead SNPs with suggestive or significant associations with NAb titers in milk*

| Chromosome | Position range (bp) | Trait | SNP and Position | MAF [1] | Genotype effect (SE) [2] | $-\log_{10}$ p-value |
|---|---|---|---|---|---|---|
| 21 | 65,998,120 – 71,599,094 | PGN-IgG1 | rs137230097 70,399,861 | 0.29 | AA: -0.35 (0.07) BB: 0.35 (0.12) | 8.1 |
|  | 69,464,920 – 71,599,094 | LTA-IgM | rs135338912 71,482,201 | 0.30 | AA: -0.17 (0.05) BB: 0.34 (0.07) | 6.9 |
| 3 |  | LPS-IgG1 | rs109075424 7,931,301 | 0.35 | AA: -0.40 (0.07) BB: 0.24 (0.11) | 7.6 |
|  | 6,266,886 – 8,339,166 | PGN-IgG1 | rs110900415 7,416,886 | 0.45 | AA: -0.21 (0.08) BB: 0.37 (0.09) | 6.3 |
| 18 | 46,339,220 – 55,991,489 | PGN-IgM | rs418923125 5,788,547 rs418923145 5,791,489 | 0.36 | AA: -0.29 (0.05) BB: 0.11 (0.07) | 6.8 |
|  | 46,339,220 – 50,839,220 | LTA-IgM | rs134833064 49,839,220 | 0.38 | AA: -0.28 (0.05) BB: 0.08 (0.06) | 6.0 [3] |
| 17 | 72,522,707 – 73,625,915 | LTA-IgM | rs133519711 73,125,915 | 0.10 | AA: 0.35 (0.06) BB: 0.04 (0.24) | 6.5 |
| 2 | 132,997,243 – 133,569,811 | LPS-IgG1 | rs110155136 133,363,107 | 0.36 | AA: -0.38 (0.08) BB: 0.17 (0.11) | 6.1 [3] |
| 29 | 10,932,414 – 15,317,753 | LTA-IgG1 | rs136539816 15,071,233 | 0.32 | AA: 0.35 (0.07) BB: -0.10 (0.08) | 5.5 [3] |
| 10 | 31,297,978 – 31,704,729 | PGN-IgG1 | rs425236353 1,497,978 | 0.22 | AA: -0.68 (0.15) BB: -0.27 (0.07) | 5.2 [3] |
| 24 | 17,964,011 – 19,750,629 | PGN-IgG1 | rs134716898 18,164,011 | 0.43 | AA: 0.06 (0.08) BB: 0.47 (0.09) | 5.2 [3] |
| 9 | 34,253,477 – 34,653,477 | LPS-IgG1 | rs435975023 4,453,477 | 0.17 | AA: -0.41 (0.08) BB: 0.09 (0.20) | 5.1 [3] |
|  | 65,759,809 – 67,772,036 | LPS-IgG1 | rs133427557 67,559,809 | 0.34 | AA: -0.27 (0.07) BB: 0.32 (0.11) | 5.3 [3] |

[1.] Minor Allele Frequency

[2.] The genotype effects are compared to the heterozygous (AB) genotype class

[3.] Suggestive association (0.05<FDR<0.20)

*IgM phenotypes*

Three significantly associated regions were found for IgM traits. The strongest association was found on BTA21 around the same region an association for IgG1 was detected from 69 to 71 Mbp. It includes 4 significant and 1 suggestive SNPs, with BovineHD2100020886 (rs135338912) located at 71,482,201 bp as the lead SNP. LTA-IgM was also significantly associated with another region on BTA17, from 72 to 73 Mbp, approximately. Only two significant SNPs were found, with lead SNP BovineHD1700021382 (rs133519711) at 73,125,915 bp.

The third region was on BTA18, associated with PGN-IgM comprised of 3 SNPs, with BovineHD1800016327 (rs41892312) and BovineHD1800016328 (rs41892314) as lead SNPs at 55,788,547 and 55,791,489 bp, respectively. The other SNP significantly associated with PGN-IgM; BovineHD1800014677 (rs134833064) at 49,839,220 bp, had a suggestive association with LTA-IgM as well.

*Candidate genes*

Candidate genes within genomic regions significantly associated with NAb titers and pathways are presented in Table 14. Genes associated with immunity and especially B cell function were selected, prioritizing genes close to the lead SNP in said region. Some regions included genes with a known role in humoral immune response but were not in any of the pathways databases; we included these genes as well.

BTA21 was the chromosome with the strongest GWAS signal. Two traits from different isotypes (PGN-IgG1 and LTA-IgM) had significant associations around the same region (65 to 71 Mbp) but different lead SNP (Table 3). For LTA-IgM, the lead SNP (rs135338912), was also the marker with the highest $-\log_{10}$ *p-values* for KLH-IgM and LPS-IgM (5.1 and 3.9, respectively), but these two traits did not reach the significance threshold. Genotype effects for rs135338912 on these three traits were additive and genotypic effects on the three traits were in the same direction. There are four immunoglobulin structural genes on BTA21 from 71.4 to 71.6 Mbp: IgE heavy chain constant region (*IGHE*), Ig heavy chain V region PJ14 (*IGHV1S20*), Ig heavy chain Mem5-like (*IGHV1S18*) and IgM (*IGHV* and *IGHV1S15* to *17*). The last three are, aside from being structural genes for IgM, according to the KEGG database also part of several immunity-related pathways such as B cell receptor signaling, NF-kappa B (**NF-κB**) signaling and PI3K-Akt signaling, so we propose them as candidate genes. In the case of PGN-IgG1, the genomic region is ~5.6 Mbp long, comprising about 70 reported genes, but only a few of these seem to be related

to antibodies. From the KEGG database, we find the genes TNF receptor associated factor 3 (*TRAF3*) and AKT serine/threonine kinase 1 (*AKT1*) both involved in Tumor necrosis factor (**TNF**) signaling and Toll-like receptor signaling. Then, *AKT1* by itself is involved in B cell receptor signaling, PI3K-Akt signaling and Fc gamma receptor-mediated phagocytosis, among others.

Table 14. *Candidate genes within genomic regions significantly associated with NAb titers in milk and selected pathways*

| Chromosome | Region (Mbp) | Trait | Gene symbol | Description | Pathway (source) |
|---|---|---|---|---|---|
| 21 | 69.5 – 71.6 | LTA-IgM | *IGHV1S20* | Ig heavy chain V region PJ14 | |
| | | | *IGHV1S18* | Ig heavy chain Mem5-like | B cell receptor signaling, NF-kappa B signaling, PI3K-Akt signaling (KEGG) |
| | | | *IGHV* and *IGHV1S15* to *17* | IgM | |
| | 66.0 – 71.6 | PGN-IgG1 | *AKT1* | AKT serine/threonine kinase 1 | B cell receptor signaling, PI3K-Akt signaling, Fc gamma R-mediated phagocytosis, TNF signaling, Toll-like receptor signaling (KEGG) Interleukin 1 to 7, 11 and 17 signaling (WikiPathways) |
| | | | *TRAF3* | TNF receptor associated factor 3 | TNF signaling, Toll-like receptor signaling (KEGG) |
| | | | *PPP2R5C* | Protein phosphatase 2 regulatory subunit B'gamma | PI3K-Akt signaling (KEGG) Negative regulator of NF-κB in TCR signaling (Breuer et al., 2014) |
| 3 | 6.3 – 8.4 | PGN-IgG1 LPS-IgG1 | *FCGR2B* | Fc fragment of IgG receptor IIb | Phagosome, Fc gamma R-mediated phagocytosis, B cell receptor signaling (KEGG) Regulation of actin dynamics for phagocytic cup formation, Fc gamma receptor-dependent phagocytosis (Reactome) |
| | | | *FCRLA* | Fc receptor like A | B cell regulation (Blackburn et al., 2017) |
| 17 | 72.5 – 73.6 | LTA-IgM | *IGLL1* | Immunoglobulin lambda-like polypeptide 1 | B cell development (Ekman et al., 2012) |
| | | | *VPREB3* | V-set pre-B cell surrogate light chain 3 | |

WikiPathways also links this gene with Interleukin (**IL**) 1 to 7, 11 and 17 signaling pathways. Another gene in this region is Protein phosphatase 2 regulatory subunit B'gamma (*PPP2R5C*) reported as part of the PI3K-Akt signaling (KEGG) and as a negative regulator of NF-κB in T cell receptor (**TCR**) signaling (Breuer et al., 2014).
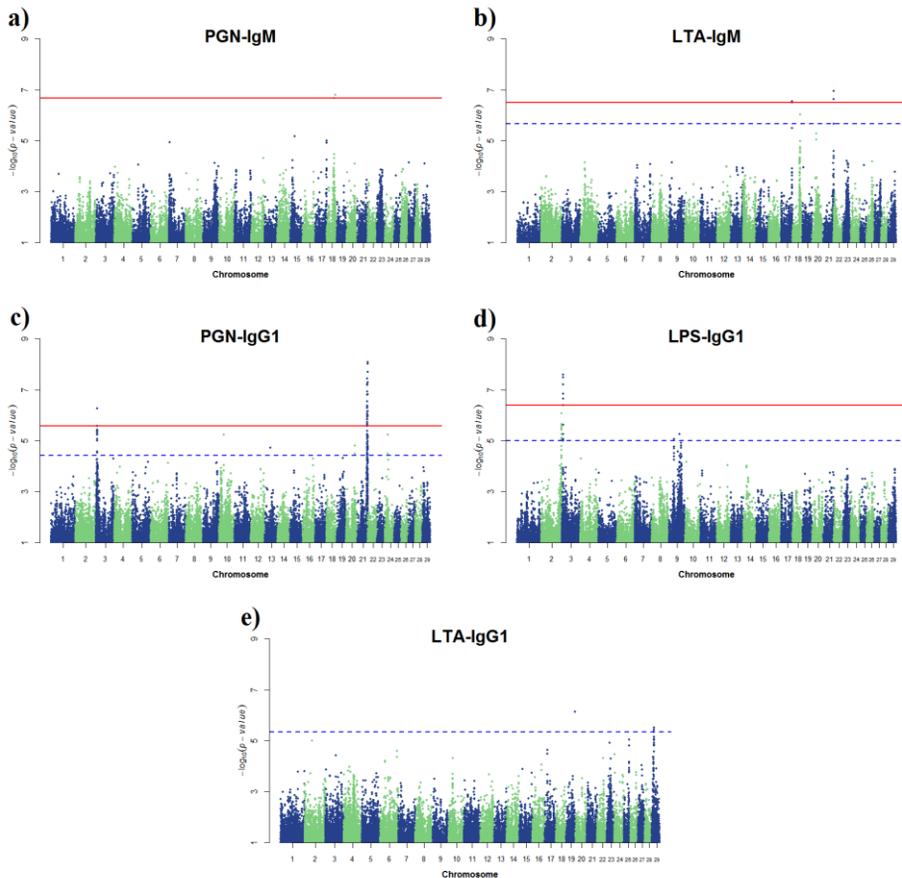


*Figure 6.* Genome-wide association study (GWAS) plots on natural antibody titers from milk **a)** PGN-IgM **b)** LTA-IgM **c)** PGN-IgG1 **d)** LPS-IgG1 **e)** LTA-IgG1. The y-axis represents the −log10(*p-value*) of the SNP association with the NAb trait. The false discovery rate (FDR) threshold was set at 0.05 for significant SNP (solid line) and at 0.20 for suggestive SNP (dotted line). For LTA-IgG1, no −log10(*p-value*) reached FDR<0.05. In the case of PGN-IgM, no SNP was 0.05<FDR<0.20 so no blue line is shown.

The second chromosome with highly significant SNPs was BTA3. On this chromosome, two IgG1 traits showed significant associations; PGN-IgG1 and LPS-IgG1. The top five SNPs for LPS-IgG1 (rs109075424, rs109851797,

rs110532702, rs43709885 and rs110858854) were also the highest for KLH-IgG1, however, significance for this trait was below the FDR<0.20 threshold. Genotypic effects for these five markers and rs110900415 (PGN-IgG1's lead SNP) were additive, and genotypic effects were in the same direction for KLH-IG1, PGN-IgG1 and LPS-IgG1. Three of these SNPs were within intronic regions of Fc fragment of IgG receptor IIb (*FCGR2B*) gene and one in the 3' untranslated region (3'-UTR). Five suggestive SNPs for LPS-IgG1 and three for PGN-IgG1 fell within Fc receptor like A (*FCRLA*) gene, spanning introns and near-gene regions. From the KEGG database, *FCGR2B* is part of the B cell receptor signaling, Phagosome and Fc gamma receptor-mediated phagocytosis pathways and according to Reactome, Regulation of actin dynamics for phagocytic cup formation. In the case of *FCRLA*, this gene is not reported in any of the databases, but it might be involved in B cell regulation (Blackburn et al., 2017).

For BTA17, only LTA-IgM had significant associations, but the top three markers with the highest $-\log_{10}$ *p-values* for this trait (rs133519711, rs135566843 and rs41854290), were also the highest for PGN-IgM and KLH-IgM (values ranged from 4.3 to 5.0), although these traits did not reach the FDR<0.20 threshold. Notably, the genotype effects for these SNPs on LTA-IgM, PGN-IgM and KLH-IgM were all in the same direction and showed complete dominance; the effect of heterozygote did not differ significantly from one of the homozygotes. None of the genes in this genomic region were reported in the databases as part of any humoral response-related pathways, however, Immunoglobulin lambda-like polypeptide 1 (*IGLL1*) and V-set pre-B cell surrogate light chain 3 (*VPREB3*) are known for playing an important role in B cell development (Ekman et al., 2012).

The genomic region in BTA18 for PGN-IgM is ~10 Mbp long due to the fact that the two lead SNPs are approximately 6 Mbp away from the rest of the significant and suggestive SNPs. There are 486 annotated genes in this region and after analyzing the region through the pathways databases, these genes were the most recurring ones among humoral response-related pathways: NFKB inhibitor beta (*NFKBIB*), AKT serine/threonine kinase 2 (*AKT2*), CD79a molecule (*CD79A*), Sphingosine kinase 2 (*SPHK2*) and Calmodulin 3 (*CALM3*). *SPHK2* was the closest one to the lead SNPs (~65 kbp), and *AKT2* was closer to the other significance peak around 49.8 Mbp (~62 kbp).

# 5 General discussion

Breeding for improved health is currently a challenge in dairy cattle. Health-related problems constitute an important economic and welfare issue that has gained more attention over the last decades. Breeding programs have started to include traits such as clinical mastitis (Heringstad et al., 2018), general health from veterinary records (Kyntäjä, 2013), and rearing period survival (Carlén et al., 2016). Selection based on immune response traits has also been implemented by some dairy cattle breeding companies (Mallard et al., 2015).

In this thesis, we have focused on the genetic component of different antibodies and indicator traits in colostrum, calf serum, and milk, aiming to reduce failure of passive transfer (FPT) through selection. We also started to investigate how these traits correlate with milk production and health.

## 5.1 Genetic parameters (Paper I)

In this study we found significant heritabilities and other variance components for most of the traits in both colostrum and calf serum, as well as phenotypic and genetic correlations between these traits.

### 5.1.1 Heritabilities

Two indicator traits were analyzed; Brix for colostrum and STP for calf serum. They both approximate antibody content by quantifying total solids (**TS**) and provide methodologically simpler but less accurate measurements compared to ELISA or radial immunodiffusion (**RID**) (Løkke et al., 2016; Elsohaby et al., 2019). Brix had a moderate heritability (0.31), which was similar to the value reported (0.27) in a previous colostrum study (Soufleri et al., 2019). In contrast, heritability for STP was not significant. This could be due to the variation from

the rest of the molecules that are co-measured, masking the antibodies' genetic variance.

Heritabilities for IgG concentration (g/L) were 0.25 and 0.20 for colostrum and calf serum, respectively. In both cases, a square root transformation was applied to normalize the residual distribution. A previous study had reported a heritability of 0.10 for colostrum IgG in Irish Holstein and crossbreeds (Conneely et al., 2013). Although the difference between our study and theirs can be explained due to being different populations in different environments, it suggests that breeding for higher colostrum IgG levels is indeed possible. In the case of calf serum, some studies in the 1980s estimated heritabilities. Gilbert et al. (1988) analyzed IgG1 in serum of 36 hour-old calves and estimated a heritability of 0.56 (0.25) using a paternal half-sib analysis accounting for the dam's colostral IgG1 concentration. In contrast, Burton et al. (1989) estimated a heritability of 0.18 (0.25) for IgG in calves 24 to 36h old using a paternal half-sib analysis.

Two antigens were used to measure NAbs: KLH and MDP. The latter is a microbial pathogen-associated molecular pattern (PAMP) comprising the minimal peptidoglycan (**PGN**) motif common to both Gram-positive and Gram-negative bacteria (Girardin et al., 2003) such as *E. coli* and *S. aureus*, which are ubiquitous in most environments. Cows in our study were not deliberately exposed to or inoculated with any of these antigens, so we regard the titers of antibodies to them as NAbs reflecting the animals' innate humoral response. Given the universal presence of MDP (PGN), it is reasonable to assume that the measurements could partly represent antibodies produced as part of the adaptive immune response. KLH on the other hand, is considered as a true measure of NAbs, since cows are not normally exposed to this antigen (van Knegsel et al., 2007). For colostrum, all NAb heritabilities were significant and for 5 out of the 6 traits ranged from 0.22 to 0.29, with the exception of KLH-IgG (0.16). This range is within the previously reported values for cow serum and milk (Wijga et al., 2013; de Klerk et al., 2015). There were no major differences between isotype heritabilities, except for KLH-IgG. In the case of calf serum NAbs, heritabilities ranged from 0.23 to 0.59. NAbs for IgA isotype had almost identical heritabilities (0.43 and 0.46) which was observed as well for IgG (0.24 and 0.26), however, there was a big difference between KLH-IgM (0.23) and MDP-IgM (0.59). This difference in IgM NAb, suggests that the way they are absorbed may be affected by the antigens they bind (or not bind to), and since IgM is larger than IgG and IgA, different transportation mechanisms may be involved.

For first test milk, NAb heritability estimates were not significant on most traits, most likely due to the limited number of samples (standard errors ranged

from 0.15 to 0.20). Other studies have estimated heritabilities of KLH NAbs in milk for different isotypes with values ranging from 0.08 to 0.48 (Wijga et al., 2013; de Klerk et al., 2015). In the case of pre-parturition serum, in spite of the large standard error, heritabilities were significant except for MDP-IgG. There are no reported heritabilities for MDP-IgG in bovine serum, but a previous study in milk (Wijga et al., 2013) estimated a value of 0.15 for PGN-IgG1.

Our results show moderate heritabilities for colostrum traits, including Brix (0.31) as an indicator, implying that genetic selection for colostrum quality is possible. Brix % has the advantage of being easier to perform than an ELISA or RID and can potentially be implemented on farms for routine assessment of colostrum quality. For calf traits, heritability estimates were moderate to high, showing that there is an important genetic component for these traits, and that the occurrence of FPT can potentially be reduced through genetic selection. Unfortunately, the heritability for STP, the indicator trait for calf serum was not significant. Providing a practical way to measure absorption of colostrum antibodies by the calf is critical to implement this trait in a breeding program.

## 5.1.2 Repeatabilities (colostrum)

Repeatability is the combined effect of genetics and permanent environmental effects. NAb in colostrum had notably higher permanent environment effects (0.10 to 0.31) than Brix (0.04) and total IgG (0.01) which made their repeatabilities proportionally higher. Permanent environment effect in this case was the effect of repeated measurements from the same cow in different parities: the more correlated the measurements for the animal across parities are, the stronger the effect. Figure 7 shows parity versus total IgG which had the smallest repeatability and KLH-IgG which had the highest. Even if parity effects have been accounted for in the model and individual changes across parities cannot be seen in the plot, it can be observed how there is no slope for KLH-IgG but there is an increasing trend for total IgG. For the latter, this is related to an accumulated exposure to antigens in older cows, which generates more specific antibodies that get transferred from the serum to colostrum (Conneely et al., 2013). NAb on the other hand, are maintained largely constant throughout life (Lutz and Miescher, 2008).
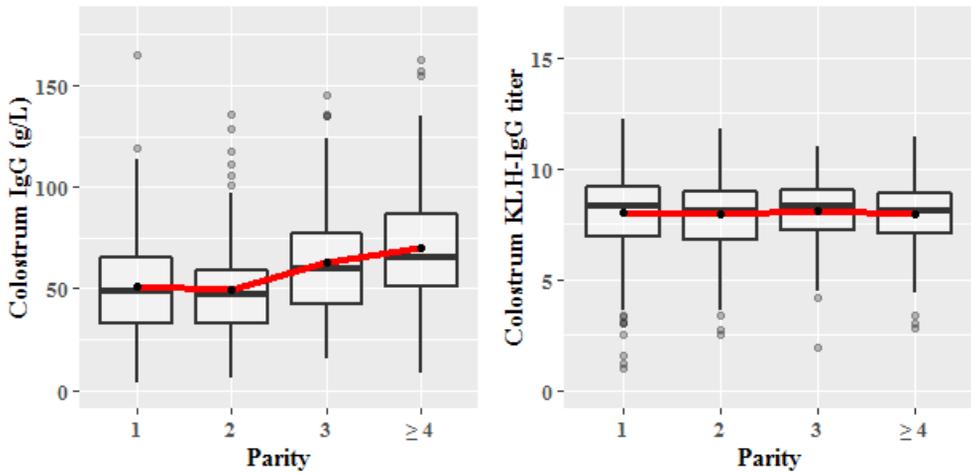
*Figure 7.* Plots of colostrum total IgG (left) and KLH-IgG titers (right) for each parity

### 5.1.3 Maternal contribution (calf serum)

Throughout the calf serum traits, the maternal contribution ranged from 17 to 37% of the variance, except for KLH-IgA, which showed no significant maternal effect. The maternal effect in this case was the non-genetic contribution of the dam across different calvings on the calf phenotype, the more similar the measurements between maternal siblings, the stronger the effect. For calf serum antibodies, we believe that the biggest (non-genetic) maternal contribution comes from the colostrum, and since we accounted for colostrum antibodies in the model, other colostrum components could be having an effect on how well the calf absorbs antibodies. Some components of colostrum are fat, protein, peptides, non-protein nitrogen, vitamins and minerals, hormones, growth factors, cytokines and nucleotides (McGrath et al., 2016). It seems plausible that some of these components differ between colostrum of different cows and directly or indirectly affect antibody uptake. One example is lactoferrin, an iron-sequestering glycoprotein with antimicrobial, anti-inflammatory, and anti-oxidative properties (Stelwagen et al., 2008), by inhibiting bacterial growth it might indirectly affect IgG uptake.

Aside from colostrum, there is increasing evidence that the calf intestine is not sterile until birth. A study by Alipour et al. (2018) found a low-abundant microbiota in rectal meconium and mucosa of calves sampled at birth resembling the composition of dam oral microbiota, but included typical intestinal taxa. The exact mechanism of how these bacteria colonize *in utero* is not clear, but these results suggest another source of maternal effect that could impact antibody uptake in the calf.

### 5.1.4 Breed

Our results show that the effect of breed is slightly higher for SLB compared to SRB or CRB in colostrum, first test milk and pre-parturition serum traits. Samples were taken on different animals, sample types and, analyzed by different techniques, but in all cases SLB had higher values. However, even for statistically significant effects, most *p-values* were barely below 0.05. Holsteins are generally assumed to have a lower colostrum quality, but several studies have found non-significant differences between Holstein and other breeds' colostrum IgG (Morrill et al., 2012; Genc and Coban, 2017; Reschke et al., 2017). Breed effects were not significant for calf serum traits except STP, for which the same pattern was seen as in colostrum.

### 5.1.5 Various effects

Time of first meal is a critical factor to avoid FPT, and in our case it was not significant. This is probably due to the fact that feedings were done within the appropriate window of time, 95% of the first meals were given before 6 hours after birth (not shown) which is the cut-off point for optimal feeding time (Godden, 2008) and significantly below the 24h cut-off.

Sampling time after birth showed a stronger negative correlation for IgM and IgA traits compared to IgG traits and STP. IgG has been shown to have a half-life of 28.5 days in colostrum-fed calves (Murphy et al., 2014), most likely because colostrum provides a fairly large amount of IgG compared to IgA and IgM isotypes, among other things. This could also explain what was seen for absolute fed colostrum (Figure 2.), where the trend was reversed, IgG traits and STP had a positive slope (~0.45) and IgA and IgM a less pronounced one (~0.32) because of the lower amount ingested.

### 5.1.6 Genetic correlations

*Colostrum traits*

Brix was significantly and positively correlated to all the other colostrum traits, ranging from 0.49 to 0.73. Several studies have pointed out the use of Brix to approximate the amount of antibodies in colostrum (Buczinski and Vandeweerd, 2016; Løkke et al., 2016), namely IgG, but to the best of our knowledge this is the first report of genetic correlation with total IgG.

Aside from Brix, total IgG was not genetically correlated with any other trait, but had a genetic correlation with KLH-IgG of 0.93 (0.23) which was strongly positive and close to being significantly different from 0 (p = 0.06). NAb traits

followed a correlation pattern observed in a previous study of NAbs in milk (Wijga et al., 2013), where IgM and IgA traits had very strong and positive genetic correlations between each other (IgM-IgA) and among the same isotype (0.82 to 0.97), suggesting a common genetic background for IgA and IgM. KLH-IgG and MDP-IgG had a correlation of 0.76, which is high but low enough to assert that they are different traits.

*Calf serum traits*

Unlike colostrum, the indicator trait for calf serum (STP), did not have significant genetic correlations with any trait. This is probably due to the non-significant genetic variance of this trait, even if the correlations have a positive tendency, the genetic covariance was not significant either. S-IgG had significant genetic correlations only with MDP-IgA and MDP-IgM and a borderline significant correlation ($p = 0.08$) with MDP-IgG. Interestingly, MDP-IgM had positive genetic correlations with all of the traits except STP and MDP-IgG, the latter having a borderline significant association. MDP-IgA also had significant or borderline significant genetic correlations with most traits except STP and MDP-IgG. Even though there is still a slightly higher genetic correlation between traits from the same isotype and between IgA and IgM, it is not as pronounced as in colostrum. This implies that isotype may play a less important role for uptake which is something that Burton et al. (1989) observed as well.

*Colostrum traits versus calf serum traits*

Measuring antibody absorption by the calf requires taking blood samples from the animal and centrifuging them to separate the serum. This means that unlike colostrum that can be sampled and measured in the farm (using a Brix refractometer), calf serum analyses require a veterinarian or technician for the sampling and a laboratory setting. For this reason, we wanted to estimate if colostrum quality in the cow correlates genetically with the calf's ability to absorb antibodies.

Originally, we attempted to correlate colostrum traits using cow for the genetic component with calf serum traits using calf for the genetic component, but there were convergence problems and correlations could not be estimated. Instead, we estimated these correlations using cow for the genetic effect on colostrum and calf serum traits. This is not ideal since the calf's genetic component comprises only half of the cow's genetics, but we wanted to get an idea of how these traits might correlate.

Only NAb IgA and IgM traits had significant genetic correlations within traits of the same isotype and between each other (IgM-IgA). It seems that only traits with very strong correlations could pass the significance threshold and even then the values had large standard errors (0.13 to 0.29). In spite of most correlations not being significant, a positive tendency could be seen for some of them, such as colostrum Brix and total IgG with calf serum IgG.

A larger sample size is necessary to estimate these correlations more accurately, and to properly correlate the colostrum traits (using cow for the genetic effect) with the calf serum traits using calf for the genetic component, instead of cow like in this case.

*Colostrum traits versus first test milk and pre-parturition serum*

Neither colostrum Brix nor total IgG were significantly correlated with any of the first test milk or pre-parturition serum NAb traits. A pattern was not apparent from the significant genetic correlations for both first test milk and pre-parturition serum NAb, this may be due to the small number of milk and serum samples used for the analysis (<300).

## 5.1.7 Production traits

*Colostrum*

To test if genetic selection for colostrum antibodies may have a negative effect on milk production, we estimated genetic correlations with milk yield, fat and protein percentage, and lactation average somatic cell score for 305 d lactation period following the calving from which the colostrum was taken. We found no significant genetic correlations between colostrum traits and milk production, although standard errors were rather high (0.13 to 0.28, mostly). Nonetheless, results suggest that there are no strong genetic correlations. Total IgG leaned towards positive correlations with fat and protein percentage, whereas Brix and protein yield seemed to have a positive trend, but a slightly negative one for fat percentage. Genetic correlation could not be estimated for Brix and LASCS, but phenotypic correlation was 0.12 (0.05).

*Calves*

There is an unclear relationship between calf serum IgG (or FPT) and milk production performance later in life. Two studies usually referenced for this topic are: a) Faber et al. (2005) who found that calves fed 2L of high quality

colostrum at birth produced significantly less milk during the first and second lactations compared to animals that were fed 4L and b) DeNise et al. (1989) who reported that for every additional g/L of IgG in serum of calves from 24 to 48 hours of age, an increase of 8.5 kg of milk and 0.24 kg of fat was observed during the first lactation. However, we were not able to find additional studies that replicate these results or even follow-ups from the same groups. Our analysis did not find significant genetic correlations between calf serum traits and milk yield, protein % or fat %.

In our study, we found significant negative genetic correlations with LASCS. KLH-IgM, MDP-IgM and MDP-IgA had correlations of -0.98, -0.96 and -0.66 respectively. Additionally, MDP-IgG, KLH-IgG and S-IgG had borderline significant negative correlations. Lactation average SCS is a log-derived measurement of somatic cell count (**SCC**), a trait that is used as a surrogate for clinical mastitis and overall udder health. LASCS has a good genetic correlation with clinical mastitis (0.6 to 0.7) (Carlén et al., 2004; Govignon-Gion et al., 2016).

We did not find significant genetic correlations of calf serum traits with AFC. KLH-IgG however, had a borderline significant negative correlation with AFC (-0.44), which is the desired direction, since increasing KLH-IgG would lead to lower AFC. Age of first calving has been described as a proxy for average daily gain (**ADG**) since a higher value leads to an earlier insemination (Raboisson et al., 2016). This result seems to be in agreement with what was found by Furman-Fratczak et al. (2011) who found that calves with higher IgG in serum had higher growth rates allowing for earlier inseminations.


## 5.1.8 Calf health traits

Health information from Röbäcksdalen calves from 0 to 3 months of age should reflect a period where maternal passive immunity still holds some effect over the calves' health. For this trait, we had 63 reported cases out of 231 animals, primarily pneumonia (70%) and diarrhea (15%), where 61 of the cases were treated with antibiotics, implying bacterial infection. This means that the binary 0/1 trait is a proxy for healthy/bacterial infection. Genetic correlations for this trait were not significant, but a negative tendency was observed for all serum traits except STP. This is a favorable trend, since the higher the antibody titer, the value for health will tend towards 0 (healthy). MDP-IgG had a borderline significant negative genetic correlation (-0.93) but with a rather large standard error (0.41). MDP is known to stimulate NOD2 which activates NF-κB (Girardin et al., 2003), so it is possible that animals with higher levels of MDP-IgG are

able to prime better the immune system by recognizing bacterial PAMPs and triggering NF-κB (Huang et al., 2019).

Data for animals from 8 to 36 months should reflect long term health effects of calf antibody levels in the animals. We had 66 cases out of 301 animals from two farms for this trait. Most cases had an unknown etiology (47%), followed by infectious (27%) and metabolic (22%). Given the small number of cases with known etiologies, it was not possible to subcategorize the trait and it was coded as 0/1 for healthy/sick. No genetic correlations were observed for any of the traits, and most trends observed were obscured by large standard errors.

Given the large standard errors observed for health traits, it might be possible to estimate more accurately these correlations with a larger number of samples.

### 5.1.9 Conclusion

We have shown that calf serum antibody traits are heritable, including S-IgG, indicating that genetic selection can be used to reduce FPT. Furthermore, there is a significant maternal contribution to calf serum antibody content beyond colostrum antibodies. Brix in colostrum has positive genetic correlations with the antibody traits investigated, thus Brix can be used as an indicator trait for selection of higher quality colostrum. Also, Brix is not genetically correlated with milk production traits in an unfavorable way. Further analyses are needed to establish the relationship between calf serum and health later in life. Our results suggest that these traits can be used for selection programs focused on genetically improving antibody content in both colostrum and calf serum.

## 5.2 Total IgG and natural antibodies in colostrum of Swedish dairy cows: a genome-wide association study (Paper II)

We have identified QTLs influencing colostrum total IgG, Brix and NAb titers using imputed 50K SNP genotypes. We found two significant regions on two chromosomes for total IgG and seven suggestive regions in seven chromosomes for different traits, but no major QTLs. Antibodies have been shown to be highly polygenic traits (Sun et al., 2013; de Klerk et al., 2018). GWAS for NAb in milk of Dutch Holstein-Friesian showed that when the SNP array is increased from 50K (Wijga, 2013) to an imputed 777K (Cordero-Solorzano et al., 2019), not only additional QTLs are found, but QTLs described in the 50K array have additional and more significant SNPs in the 777K array, which may be useful for genomic selection. In our case it seems probable that we may have missed

some QTLs because of the density of our SNP array and that we may not have found the most significant SNPs for our QTLs.

Two antigens were used to measure NAbs: KLH and MDP. The latter is a microbial pathogen-associated molecular pattern (PAMP) comprising the minimal peptidoglycan (**PGN**) motif common to both Gram-positive and Gram-negative bacteria (Girardin et al., 2003) such as *E. coli* and *S. aureus*, which are ubiquitous in most environments. Cows in our study were not deliberately exposed to or inoculated with any of these antigens, so we regard the titers of antibodies against them as NAbs reflecting the animals' innate humoral response. Given the universal presence of MDP (PGN) it is reasonable to assume that the measurements could partly represent antibodies produced as part of the adaptive immune response. KLH on the other hand, is considered as a true measure of NAbs, since cows are not normally exposed to this antigen (van Knegsel et al., 2007).

Genetic correlations between IgM and IgA NAb in milk and colostrum are very strong and larger than 0.8 (Paper I and Wijga et al., 2013), but we only found one QTL influencing IgA and IgM traits simultaneously. In general, our results show limited pleiotropy, but this could be due to a limited statistical power.

To the best of our knowledge, only two other GWAS studies have focused on antibodies in colostrum. Berry et al. (2013) found haplotypes of polymeric immunoglobulin receptor gene (*PIGR*) significantly associated with IgA content in colostrum of Holstein-Friesian x Jersey crossbreds. *PIGR* is located around 4.5 Mbp on BTA16, but we did not find any association in this region. It is possible that this gene may not have variation that affects IgA significantly in our population, but it also seems plausible that since their samples were taken during second milking colostrum instead of first milking like our samples, the effect of *PIGR* became more evident or different genes are involved. The other study was from Wagter-Lesperance et al. (2018) on colostrum IgG of Canadian Holsteins, they found two suggestive QTLs; one on BTA1 and another on BTA9, but we could no replicate their results. Recently, Kiser et al. (2019) reported a QTL on BTA3 for Brix on colostrum, in the same region that we found a suggestive association for Brix and MDP-IgM.

## 5.2.1 Role of candidate genes

*IgG phenotypes*

On BTA11 for total IgG, *CD8A* and *CD8B* were found as candidate genes. CD8a and b code for the cluster of differentiation of CD8+ T cells, which in colostrum

are potent interferon-gamma (IFNγ) producers, playing an important role in cellular immunity by contributing to microorganism elimination (Hagiwara et al., 2008). Given the diverse and complex functions of IFNγ, the exact role these genes might play needs to be studied further.

On chromosome 20, in the region around 52 Mbp, we found a significant association with total IgG and a suggestive association with MDP-IgG, in this same region, de Klerk et al. (2018) found an association for KLH-IgG in cow serum and in Paper III we found an association for KLH-IgM in calf serum. Candidate gene myosin X (*MYO10*) is associated with Fc gamma receptor (*FCGR*) mediated phagocytosis (KEGG). In this same region, cadherin 18 (*CDH18*) is related to cell-cell junction organization and adherens junctions interactions (Reactome). The role these genes might play is not clear.

*IgM and IgA phenotypes*

On BTA3 we found suggestive associations for Brix and MDP-IgM. Vascular cell-adhesion molecules are involved in the migration of T and B cells into the mammary gland. Hodgkinson et al. (2007) reported that *VCAM1* was strongly expressed at the mammary alveolar tissues during the colostral phase. It decreases at the lactation phase and afterwards it is not detectable, suggesting a role in the active migration of cells into colostrum for transfer to the neonate, prior to gut closure. Additionally, in this same region, Kiser et al. (2019) found a significant association for colostrum Brix in Jersey and (Leach et al., 2012) for serum IgG2 against bovine respiratory syncytial virus.

On chromosome 7, we found an association around 74 Mbp for both KLH-IgM and MDP-IgA, with candidate gene Interleukin 12B (*IL12B*). This gene codes for the p40 chain of Interleukin 12, a pro-inflammatory cytokine.

## 5.2.2 Conclusion

We have found regions associated to different antibody traits and Brix in colostrum, but no major QTLs were found. A higher density array or whole-genome sequence variants might help find markers with stronger effects.

Candidate genes found are related to immunity and leukocyte migration, but further studies are needed to understand how their functions relate to colostrum quality.

## 5.3 Genomic regions associated with antibody uptake in newborn calves (Paper III)

In this study, we performed GWAS on different antibody traits and indicators for calf serum. To the best of our knowledge this is the first genome-wide analysis on such traits.

We identified QTLs influencing calf serum total IgG, and NAb titers using imputed 50K SNP genotypes. Five genomic regions were significantly associated on five different chromosomes and nine regions had suggestive associations on eight other chromosomes, but no major QTLs were found. In this case, we are looking at the calf's antibody absorption capabilities and not its antibody production capacity. Based on the GWAS results it appears that these traits are highly polygenic, so there is a possibility that using a higher density SNP array might help finding additional QTLs or markers with higher significance.

Aside from S-IgG, two antigens were used to measure IgG, IgA and IgM NAbs: KLH and MDP. The latter is a microbial pathogen-associated molecular pattern (PAMP) comprising the minimal peptidoglycan (**PGN**) motif common to both Gram-positive and Gram-negative bacteria (Girardin et al., 2003) such as *E. coli* and *S. aureus*, which are ubiquitous in most environments. Some studies suggest that antibodies in the calf's gut are absorbed in a semi-selective way, but the exact mechanisms are not known (Stott et al., 1979; Jochims et al., 1994). High genetic correlations ($> 0.6$) that we found in Paper I for NAb regardless of isotype and S-IgG in calf serum seems to confirm this, or at least that the mechanisms are not isotype-specific. Given this, it is surprising the limited pleiotropy observed; only one QTL was associated with two traits at the same time and they both were IgG, this could be due to a limited statistical power.

A previous study (Laegreid et al., 2002) reported haplotypes of bovine neonatal Fc receptor α-chain gene (*FCGRT*) having an effect on neonatal serum IgG concentration. This gene is located around 56.4 Mbp on BTA18, but we could not find an association in this region or nearby. One explanation could be that the polymorphisms in our population for this gene do not affect IgG concentration, or that the SNP chip does not cover the region well enough to detect significant variation. From our SNP array, there is one marker located in the gene locus (HAPMAP56958 [rs41256930]) with a MAF of 0.22 in our population, but as a synonymous variant. Using a higher density array might help finding an association for this gene, however, it is apparent from our results that genes with very large effects on these traits are not segregating in this population. There might be QTLs with smaller effects that we are not detecting, but will only be detected on a larger sample.

### 5.3.1 Role of candidate genes

Our GWAS shows two regions containing claudin genes, one in BTA1 for claudin 8 (*CLDN8*) and claudin 17 (*CLDN17*) and another in BTA27 for putative claudin 24 (*LOC112444630*) and claudin 22 (*LOC536739*). These genes may play a role in the intestinal permeability. Tight Junction (TJ) is the structural feature that helps maintain a controlled separation of the gut content from the rest of the body. Structurally, TJ is made up of four different integral proteins of which claudins are one of them. Claudins have a fundamental role in barrier function, they can seal or make pores within the gastrointestinal tract (Liang et al., 2016).

Candidate genes for both regions on BTA4 might be related to gastric acid secretion. *VIPR2* is associated with water and ion regulation in the intestine as its ligand, vasoactive intestinal peptide (**VIP**) inhibits gastric acid secretion, but stimulates biliary water and bicarbonate secretion (Gozes and Furman, 2004). For *ADCY1* and *CAMK2B* according to KEGG database they are part of the gastric secretion pathway. Regulating secretion of gastric acid may have an effect on antibody uptake, it seems reasonable to assume that an overly acidic gut during the first 24 hours of the calf can have a detrimental effect by denaturing or breaking down antibodies.

On BTA10, *DOUX2* is a candidate gene present in the salivary glands and gastrointestinal tract. Its production is stimulated by MDP and it is related to NOD2 signaling (Al Nabhani et al., 2017). NOD2 has been shown to be involved in the regulation of innate and adaptive immune responses and more recently with the regulation of microbiota in the small intestine of humans (Sidiq et al., 2016).

For BTA20 around 52 Mbp, we found an association for KLH-IgM, the same region found by de Klerk et al. (2018) for KLH-IgG in cow serum and in Paper II for T-IgG and MDP-IgG in colostrum. The role of the proposed candidate genes might play is not clear. But the fact that this QTL has been found in three different antibody traits in two different populations, indicates some degree of pleiotropy.

### 5.3.2 Conclusion

We have found regions associated to different antibody traits in calf serum, but no major QTLs were found. A higher density array or whole-genome sequence variants might help find markers with stronger effects.

Candidate genes found are related to immunity, gastric acid and saliva secretion, leukocyte migration and tight junction, but further studies are needed to understand how they relate to calves' antibody absorption.

## 5.4 Genome-wide association study identifies loci influencing natural antibody titers in milk of Dutch Holstein-Friesian cattle (Paper IV)

In the present study, we identified QTLs influencing NAb titers using imputed 777K SNP genotypes. We found five significant regions on four chromosomes and six suggestive regions on five other chromosomes associated with IgG1 and IgM NAb titers in milk.

Four antigens were used to measure NAbs: KLH, LPS, LTA and PGN. The last three are microbial PAMPs found on bacteria such as *E. coli* and *S. aureus* (Baumgarth et al., 2005), which are ubiquitous in most environments. Cows in our study were not deliberately exposed to or inoculated with any of these four antigens, so we regard the titers of antibodies to them as NAbs reflecting the animals' innate humoral response. Given the universal presence of LPS, LTA and PGN it is reasonable to assume that the measurements could partly represent antibodies produced as part of the adaptive immune response. KLH on the other hand, is considered as a true measure of NAbs, since cows are not normally exposed to this antigen (van Knegsel et al., 2007).

No significant associations were found for any of the KLH NAb isotypes, but there were some cases in which the lead SNP in a chromosome for a significant trait was also the lead SNP for KLH. This was the case for KLH-IgM with LTA-IgM on chromosomes 17 and 21, as well as KLH-IgG1 with LPS-IgG1 on chromosome 3. A possible explanation might be that these genomic regions comprise genes related to innate immune response or shared between innate and adaptive immune response.

There were also no associations found for any IgA or total NAbs traits. For the latter, this could be due to a dilution effect, the trait includes all isotypes at the same time, which are mostly regulated by different genetic mechanisms, creating additional noise in the GWAS signal. For IgA the results are quite intriguing, since genetic correlations with IgM NAbs are very strong and larger than 0.8 (Wijga et al., 2013). It is possible that the SNP genotypes do not cover genomic regions involved in shared regulatory mechanisms between both isotypes. Further analyses with IgA NAbs are required to better understand this.

A previous GWAS on bovine NAbs (de Klerk et al., 2018) identified genomic regions of interest. They analyzed NAbs in Canadian Holstein cows' serum using a 50K SNP array and found a region on BTA21 from 55.5 to 70.6 Mbp significantly associated with KLH-IgG, overlapping with our region from 66.0 to 71.6 Mbp for PGN-IgG1. Therefore our results confirm findings by de Klerk et al. (2018), which is remarkable as there are important trait differences between both studies: our samples were taken from milk whereas theirs were from serum, we analyzed IgG1 (an isotype subclass) and they analyzed IgG (which includes

all isotype subclasses) and the antigens used were also different. It has been shown that there is a high genetic correlation between NAbs of the same isotype in serum and milk; 0.79 (±0.09) for IgM and 0.81 (±0.18) for IgG (de Klerk et al., 2015). Also, IgG1 is the predominant subclass in both serum and milk (approximately 56% and 74%, respectively) (Marnila and Korhonen, 2011). This implies that even if KLH-IgG from serum and PGN-IgG1 in milk are different traits, it is reasonable to assume that they share genetic regulatory mechanisms.

The other genomic regions we found, have not been reported in other NAb or SpAb studies in cattle, as far as we know.

## 5.4.1 Role of candidate genes

*IgM*

B cell development involves different stages and checkpoints before moving on to the next phase. The pro-B stage requires rearrangement and expression of immunoglobulin heavy-chain (**Ig H**) precursors (including *IGHV* gene family [BTA21]) (Spanopoulou et al., 1994). After that, at the pre-B cell stage the production of surrogate light-chain (**SLC**) (encoded by *IGLL1* [BTA17] and *VPREB1*) is required (Mundt et al., 2001). Then, SLC assembles with Ig H to form the pre-B cell receptor (**pre-BCR**), temporarily substituting the actual immunoglobulin light-chain (**Ig L**). When this receptor assembles, the cells proliferate and later on rearrange and produce Ig L, to form a B cell receptor (**BCR**) (Almqvist and Mårtensson, 2012).

How well Ig H associates with SLC and the reactivity of BCR with self-antigens affect cell survival, ultimately having an important effect on B cell repertoire (Melchers et al., 2000; Herzog and Jumaa, 2012). Our proposed candidate genes for IgM NAbs (BTA17 and 21) may have an independent influence, but both during early stages of development.

*IgG1*

Fc receptors (**FcR**) are molecules on the surface of cells that recognize and attach to the Fc region of immunoglobulins, playing a role in the regulation of innate and adaptive immunity. These receptors are expressed in several immune cells such as macrophages, neutrophils and others (Moldt and Hessell, 2014). Interestingly, there is only one FcR known to be expressed on B cells; FcγRIIb, which binds to IgG antibodies and is encoded by the gene *FCGR2B* (BTA3). This is a negative regulator that prevents overstimulation of B cells and

activation of low-affinity, self-reactive BCRs (Walsh and Bolland, 2014). A study on human immunoglobulin levels linked *FCGR2B* with total IgG concentration. Mutation analyses revealed a positive effect through loss of function (Jonsson et al., 2017), since FcγRIIb receptor suppresses IgG production (Hogarth and Pietersz, 2012). This is consistent with our results for PGN-IgG1 and LPS-IgG1 on BTA3 where genotype effects show an additive pattern (Table 3), however, the alternative allele had a positive effect.

In this same region, we found another candidate gene that belongs to the family of Fc receptor like (**FcRL**) molecules; *FCRLA3*. This receptor is a soluble protein located in the endoplasmic reticulum (**ER**) that can bind to multiple Ig isotypes (Wilson et al., 2010; Reshetnikova et al., 2012). Although its exact role is still unknown, it has been speculated that it may enhance stimulation from Th cells, facilitating class switching (Blackburn et al., 2017).

On chromosome 21, TRAF3 is an adapter protein expressed in several immune cells (including B cells) and plays many different roles in immune function regulation. Jonsson et al. (2017) found an association with this gene and class switching for IgA and IgG, confirming a previous study that showed TRAF3 plays a key role in CD40-mediated class switching (Jabara et al., 2009). This gene has also been shown to negatively regulate homeostatic survival of B cells (Moore et al., 2012) and to play an essential role in T cell-dependent IgG1 production (Xie et al., 2011), among others. *TRAF3* is in the genomic region that overlaps with the QTL found by de Klerk et al. (2018) and it is also one of their proposed candidate genes.

As for *PPP2R5C*, it expresses the regulatory subunit B56γ of Protein phosphatase 2A (**PP2A**). This enzyme has been shown to suppress the activation of NF-κB in T cells (Breuer et al., 2014). PPP2R5C is also a modulator of liver metabolism, constituting an important factor in energy metabolism and maintenance of a metabolically healthy state (Cheng et al., 2015). De Klerk et al. (2015) found that NAb levels are indicators of potential problems after calving related to Negative Energy Balance (**NEB**). Thus, this gene suggests a link between NAbs and health status through liver metabolism.

Another candidate gene in this region, *AKT1*, is part of the PI3K-AKT pathway which plays a central and diverse role in many cell types, involving cell growth, proliferation and survival. The immunological functions of AKT1 appear to be rather broad and still not fully understood. Some of the known functions include: T cell development (together with AKT2) (Juntilla et al., 2007) and FOXO1 signaling which involves maturation and survival of peripheral B cells and class switching (Calamito et al., 2010; Chen et al., 2010).

It seems plausible that variation in these candidate genes associated with IgG1 titers, might have an effect on class switching via different but related

mechanisms involving Th cells. This could imply that the observed effects on IgG1 are more related to an adaptive humoral response rather than natural antibodies.

## 5.4.2 Conclusion

Natural antibodies in milk have an important genetic component and we identified specific variants in the genome that influence NAbs. Candidate regions for IgM traits point towards immunoglobulin structural genes and early B cell development which plays a crucial role in the B cell repertoire. As for IgG1 traits, the associated genes appear to be related with class switching involving Th cells, hinting that the observed effects might be part of an adaptive response. Our results provide new insights into the regulation of milk NAbs which will help to unravel the complex relation between milk immunoglobulins and disease resistance in dairy cattle. A better understanding of these relationships is needed before milk NAbs can be used as a biomarker for management or as a selection criterion

# 6   Conclusions

The conclusions of this thesis can be summarized as:

➢ Antibody uptake traits of newborn calves have moderate heritabilities and selective breeding could be used to reduce FPT. This would require extensive phenotyping of newborn calves.
➢ The maternal contribution to calves serum antibodies is more than just the colostrum antibodies.
➢ Calf serum KLH-IgM, MDP-IgM and MDP-IgA had negative (favorable) genetic correlations with LASCS during first lactation, so potentially they can be used as early indicator traits to select against mastitis.
➢ Calf serum traits and health from zero to three months of age had a negative tendency (favorable) in genetic correlations, but these were not significant. Increasing the number of samples and separating diseases by etiology might help to clarify these relationships.
➢ Colostrum traits from the cow may potentially be used to predict the calves' ability to uptake colostrum, our analysis using the genetic component of the cow to correlate both traits suggests it might be possible, but further analysis with the calves' genetic component are needed to better elucidate this.
➢ Colostrum antibody traits have moderate heritabilities and Brix is genetically correlated with total IgG and NAb, so it can be used as an on-farm indicator trait to select for better colostrum quality.
➢ No strong QTLs were found for colostrum or calf serum antibody traits, higher density arrays are needed to scout for markers with stronger associations. Alternatively a much larger number of animals needs to be assayed to enable genomic selection.
➢ Based on the imputed GWAS on the Swedish animals and the high density GWAS on the Dutch animals we can postulate that we most likely only identified a fraction of the QTL in the Swedish data.

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# Popular science summary

The first few weeks of a calf's life are critical. Most of the deaths happen during this period and animals who get sick at this point usually do not perform as well later in life. Colostrum is the first milk produced immediately after delivery of the calf and contains both antibodies to protect the newborn and a higher concentration of protein and fat. Colostrum of good quality and proper management can prevent death of calves and improve their health. Yet this aspect of calf rearing is often overlooked by animal breeders.

We have found that it is possible to measure colostrum quality in a practical way using a Brix refractometer. These measurements allow to improve colostrum quality through genetic selection, which in turn can translate into cows that produce colostrum of more consistent quality in the farms. Additionally, we found that the calf's own capacity to absorb the colostrum's protective elements can also be selected for genetically. Overall, our findings suggest that including these traits in breeding programs may provide a better health and later performance of the calves, increasing the farm's welfare and profitability.

# Populärvetenskaplig sammanfattning

För kalvar är dem första veckorna i livet kritiska. De flesta dödsfall inträffar under den här perioden och djur som blir sjuka tidigt i livet presterar vanligtvis inte lika bra senare. Råmjölken, den första mjölken som kon producerar direkt efter kalvens födsel, innehåller både antikroppar som skyddar den nyfödda kalven från infektioner samt en högre koncentration av protein och fett. Råmjölk av bra kvalité och rätt skötsel kan förebygga dödsfall bland kalvar och förbättra deras hälsa, men den här aspekten glöms ofta bort vid uppfödning av kalvar.

Vi har upptäckt att det är möjligt att mäta kvalitén av råmjölken på ett enkelt sätt genom att använda ett så kallad Brix refraktometer. Den här typen av mätning möjliggör en förbättring av råmjölken genom genetisk selektion vilket i sin tur innebär att korna på gården kan producera råmjölk av mer jämn kvalité. Vi har också upptäckt att det är även möjligt att selektera för kalvens förmåga att absorbera råmjölkens skyddande faktorer. Sammantaget har vi kommit fram till att en inklusion av dessa egenskaper i avelsprogram kan innebära bättre hälsa och prestation senare i livet för kalvarna. Detta förbättrar även gårdens välfärd och lönsamhet.

# Resumen de divulgación científica

Las primeras semanas de vida de un ternero son críticas. La mayoría de las muertes ocurren durante este período y los animales que se enferman en este punto generalmente no se desempeñan tan bien más adelante. El calostro es la primera leche producida por la vaca inmediatamente después del parto y contiene anticuerpos que brindan inmunidad pasiva al ternero recién nacido y una mayor concentración de proteínas y grasas. El calostro de buena calidad y su manejo adecuado pueden prevenir la muerte de terneros y mejorar su salud. Sin embargo, este aspecto de la crianza es a menudo ignorado por los criadores de animales.

En nuestra investigación hemos descubierto que es posible medir la calidad del calostro de manera práctica utilizando un refractómetro Brix. Estas mediciones permiten mejorar la calidad del calostro a través de la selección genética, con vacas que produzcan un calostro de calidad más consistente en las granjas. Además, hemos descubierto que la capacidad del ternero para absorber los elementos protectores del calostro también se puede seleccionar genéticamente. En general, nuestros hallazgos sugieren que la inclusión de estos rasgos en los programas de reproducción puede proporcionar una mejor salud y un mejor rendimiento para los terneros, aumentando el bienestar animal y rentabilidad de la granja.

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