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GENETIC AND GENOMIC BACKGROUND OF LACTOSE IN BOVINE MILK

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To Gianluca and Yosef

Propositions

Because this is a genetic problem, there is obviously a genetic solution.
(Jennie Pryce, 2014)

Genome editing and promotion of alleles by genome editing have the potential to transform animal breeding. Its effectiveness will depend on societal acceptance and our ability as a community to overcome current technological hurdles.
(John Hickey and collaborators, 2016)

Lactose content could be a potential additional trait for inclusion in udder health indexes along with somatic cells. Breeding for lactose makes sense in this perspective.
(this thesis)

We are only at the beginning of generating large-scale data in those fields, but integrating those results with genomic data will strongly improve our biological understanding of genetic causes and mechanisms underlying relevant phenotypes, allowing more knowledge-based breeding strategies to be developed.
(Noelia Ibanez-Escriche and Henner Simianer, 2015)

“Best” is a relative term. There is no best animal for all situations. The kind of animal that works best in one environment may be quite different from the best animal under another set of circumstances.
(Piter Bijma and Johan A. M. van Arendonk, 2004)

In the era of big data, modern hi-tech milking systems can provide daily information about milk from individual cows; this would allow for a better understanding of the phenotypic and genetic “behavior” of milk lactose within and across lactations.
(this thesis)

La scelta di un giovane dipende dalla sua inclinazione, ma anche dalla fortuna di incontrare un grande maestro.
(Rita Levi Montalcini)

Declaration

I declare that the present thesis has not been previously submitted as an exercise for a degree at University of Padova (Italy) or any other University.

I further declare this work embodied to be mine.

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Abstract

Milk lactose has recently gained interest in the scientific community thanks to its association with udder health and the need of novel indicators for cattle breeding. Moreover, there is economic interest behind solid lactose worldwide for manufacturing purposes, especially food and pharmaceutical industry. This is fully demonstrated by the increased number of papers dealing with lactose in the last decade. Most publications report lactose percentage (LP) to be negatively correlated with milk somatic cell count (SCC), used as indicator of mastitis together with its score (SCS). On the other hand, lactose yield (LY) directly determines milk volume.

The overall aims of the present thesis were: i) to identify sources of variation of LP and LY; ii) to assess genetic parameters of LP and LY; iii) to estimate phenotypic and genetic associations of LP and LY with traditional traits (milk yield, fat percentage, and protein percentage), milk coagulation properties, freezing point, and minerals content; iv) to estimate genetic correlations of LP and LY with mastitis and other common health disorders; and v) to identify genomic regions coding for LP and LY, and search for overlaps with regions coding for SCS and mastitis.

Chapter 1 provides an overall picture of phenotypic and genetic variation of LP and LY in Italian Holstein breed, and of their associations with traditional milk-related traits, including SCS. In Chapter 2, the relationships of LP and LY with milk freezing point, minerals and coagulation properties predicted from milk mid-infrared spectra are presented and discussed. Chapter 3 deals with estimation of genetic correlations of LP and LY with major health disorders of dairy cows diagnosed by veterinarians, namely mastitis, ketosis, milk fever, retained placenta, and ovarian cysts. Finally, results of the genome-wide association study carried out for LP and LY are presented in Chapter 4.

Based on the results of the present thesis, LP is a potential indicator of udder health and mastitis resistance in dairy cattle. However, due to limited variability of LP at population level, some derived traits, e.g. based on lifetime LP trend in cow, should be further investigated and exploited in order to maximise response to selection in the long term.

Riassunto

Nella comunità scientifica, l'interesse per il lattosio del latte bovino è emerso solo in tempi recenti, grazie alla correlazione con la salute della mammella e al sempre più crescente bisogno di caratteri alternativi come nuovi indicatori per fini selettivi. Inoltre, in alcuni mercati, il lattosio in forma solida ha un valore economico elevato grazie all'utilizzo nell'industria alimentare e farmaceutica. Negli ultimi anni si è osservato un crescente numero di pubblicazioni riguardanti la correlazione fra la concentrazione di lattosio (LP) e le cellule somatiche del latte. Queste ultime sono infatti uno degli indicatori di mastite più diffusi e utilizzati al mondo, sia sotto forma di conta di cellule (SCC), sia come punteggio (SCS). Per quanto concerne la produzione giornaliera di lattosio (LY), questa risulta responsabile della quantità di acqua presente negli alveoli e quindi del volume di latte prodotto.

Gli obiettivi di questa tesi sono stati: i) caratterizzare fenotipicamente LP e LY identificando le maggiori fonti di variazione; ii) stimarne i parametri genetici; iii) calcolare le correlazioni fenotipiche e genetiche con la produzione di latte, la concentrazione di grasso e proteina, le proprietà coagulative, l'indice crioscopico e il contenuto minerale del latte; iv) stimare le correlazioni genetiche di LP con caratteri di salute rilevati da veterinari, incluse mastite e chetosi; v) identificare le regioni del genoma bovino che influenzano in maniera significativa LP e LY, comparandole con quelle identificate per mastite e SCS in altri studi.

Il Capitolo 1 fornisce una panoramica delle principali fonti di variazione di LP e LY, e presenta le correlazioni genetiche e fenotipiche con produzione di latte, percentaule di grasso, percentuale di proteina e SCS. Nel Capitolo 2 vengono considerati alcuni caratteri maggiormente legati ad aspetti tecnologici del latte: indice crioscopico, proprietà coagulative e contenuto dei maggiori minerali presenti. Le correlazioni di LP e LY con le maggiori patologie che interessano la vacca da latte (mastite, chetosi, febbre da latte, ritenzione di placenta e cisti ovariche) sono presentate nel Capitolo 3. Infine, i risultati ottenuti dallo studio di associazione *genome-wide* sono riportati nel Capitolo 4.

I risultati del presente lavoro di tesi hanno fatto emergere il potenziale ruolo di LP come indicatore di salute della mammella e di resistenza alla mastite nella bovina da latte. Tuttavia, considerando la variabilità limitata del carattere, dovranno essere

studiati dei caratteri derivati da LP, magari sfruttando l'andamento di LP durante il corso della vita della bovina. Questi caratteri, se informativi, variabili ed ereditabili, potranno essere considerati validi candidati per massimizzare la risposta alla selezione nel medio-lungo periodo.

General introduction

Lactose is one of the major solids of bovine milk and its concentration averages 4.80%. Chemically, lactose is a disaccharide made of glucose and galactose and its sweetness is a quarter that of sucrose. Thanks to its favourable technological characteristics, this sugar is commonly powdered and used for food and drug manufacture worldwide. Lactose percentage (LP) of bovine milk scarcely varies within lactation, compared with fat and protein percentage, and weakly correlates with milk yield (MY) and fat and protein percentages. On the other hand, lactose yield (LY) directly defines the amount of MY, by acting as the main osmole in the alveolar structures of mammary gland and drawing the water from blood. Both phenotypic and genetic correlations between LY and MY are thus close to unity. Lactose is strictly related to udder environment and LP is reported to be negatively related to somatic cell score (SCS), the most popular indicator of mastitis and udder inflammation. In particular, LP tends to reduce in cows suffering from mastitis and/or showing high milk SCS; both the phenotypic and genetic correlations are in the order of -0.30 in cosmopolitan dairy breeds. The negative association in infected mammary glands is due to an increased permeability of epithelium caused by weak tight junctions of cells; this simultaneously causes a loss of lactose which is then found in the bloodstream and urines, and reduces LP in the alveolar structures, i.e. in the milk. This explains why LP and LY are weakly correlated to each other and differently correlate with other milk characteristics and health traits.

Although lactose is usually more abundant than fat and protein, it has not been investigated as in the case of the other traits due to its low variability and economic value. However, in some countries such as New Zealand, lactose gained importance in the last decade, in conjunction with a significant increase of production and export of dairy powders. Moreover, an increased number of publications dealing with LP and/or LY has been observed from 2000 until now, mainly focusing and investigating the relation between LP and SCS.

A better understanding of the relationship between LP and SCS is needed in order to validate lactose as proper indicator for genetic purposes to design selection schemes aimed at improving udder health and mastitis resistance in dairy cattle. In fact,

according to selection index theory, indicators have to be more heritable than the real objective (the breeding goal), show genetic variability, and correlate with the objective trait.

Invited review: Milk lactose - Current status and future challenges in dairy cattle

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Invited review: Milk lactose—Current status and future challenges in dairy cattle

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ABSTRACT

Lactose is the main carbohydrate in mammals' milk, and it is responsible for the osmotic equilibrium between blood and alveolar lumen in the mammary gland. It is the major bovine milk solid, and its synthesis and concentration in milk are affected mainly by udder health and the cow's energy balance and metabolism. Because this milk compound is related to several biological and physiological factors, information on milk lactose in the literature varies from chemical properties to heritability and genetic associations with health traits that may be exploited for breeding purposes. Moreover, lactose contributes to the energy value of milk and is an important ingredient for the food and pharmaceutical industries. Despite this, lactose has seldom been included in milk payment systems, and it has never been used as an indicator trait in selection indices. The interest in lactose has increased in recent years, and a summary of existing information about lactose in the dairy sector would be beneficial for the scientific community and the dairy industry. The present review collects and summarizes knowledge about lactose by covering and linking several aspects of this trait in bovine milk. Finally, perspectives on the use of milk lactose in dairy cattle, especially for selection purposes, are outlined.
Key words: lactose, bovine milk, health trait, breeding, dairy industry

INTRODUCTION

Milk is an energetic animal-derived food, rich in essential fatty acids, vitamins, minerals, amino acids, and oligosaccharides. Among these nutrients, lactose is the main sugar, uniquely found in mammals' milk. Lactose contributes to the energy value of milk, as reported in the formula of Tyrrell and Reid (1965): total energy output (MJ/kg) = [0.384 × fat percentage + 0.223 × protein percentage + 0.199 × lactose percentage − 0.108] × milk yield (kg).

In bovines, lactose is the major milk solid (Fox et al., 2015), and it is individually recorded in lactating cows almost all over the world under routine evaluation systems. In recent decades, likely due to the increased availability of milk data from infrared predictions, lactose percentage (**LP**) has been included in scientific studies and reports, together with traditional traits such as milk yield, fat percentage, and protein percentage. Although LP has been considered a low-informative trait for decades due to its low variability, some investigations have reported interesting findings, particularly its negative relationship with SCC. Thus, interest in LP has increased, as has the number of published papers dealing in some manner with lactose (Figure 1). Nevertheless, there is no uniform consensus about the phenotypic and genetic factors affecting LP and lactose yield (**LY**). After the optimization of dairy industry efficiency and the application of whey filtration technologies, lactose powder has become a food ingredient with a market demand and value (CLAL, 2018). In spite of this, scientific knowledge about the physiology and variability of lactose is still scarce, because of consensus that this compound is constant in milk and does not affect milk quality and technological properties. However, some studies have demonstrated that a certain informative variation in lactose within and across lactations exists in cattle (Miglior et al., 2007; Alessio et al., 2016; Costa et al., 2018). Therefore, it seems appropriate to provide a clearer, more complete picture for lactose, to

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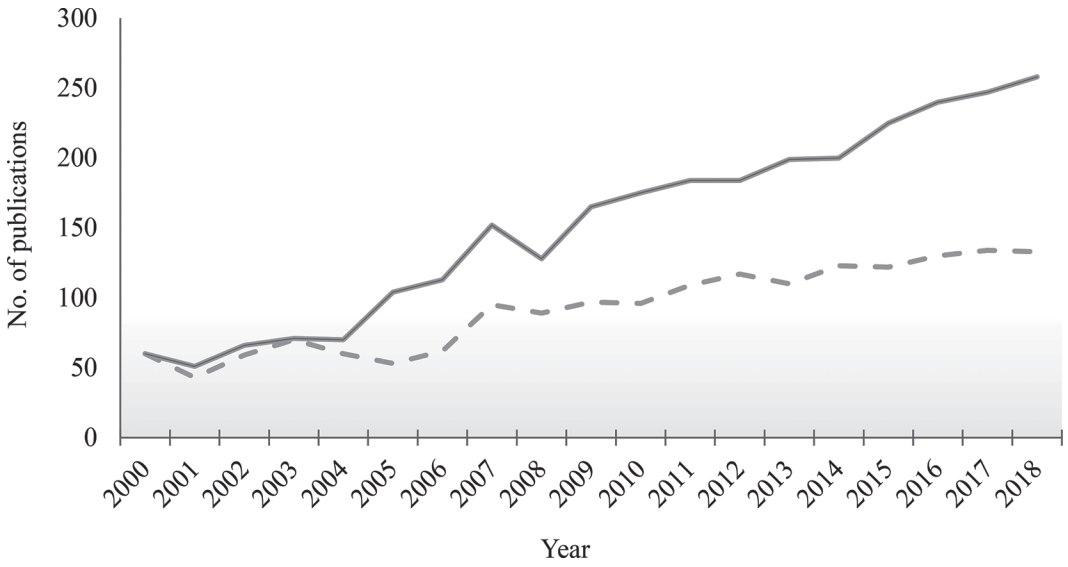

Figure 1. Number of published articles, reviews, and conference papers that cite the key words "lactose, dairy" (solid line) or "lactose, cow, milk" (dashed line) in the title or in the abstract. Source: Scopus, www.scopus.com.

understand if and how this trait can be exploited in the dairy field.

SYNTHESIS AND CHEMICAL FORMS OF MILK LACTOSE

Biosynthesis Pathways

Lactose is synthesized in the udder from blood glucose absorbed by the basal membrane of mammary epithelial cells (Osorio et al., 2016). Around 20% of the circulating blood glucose of a dairy cow is converted into lactose during lactation (Cant et al., 2002; Rigout et al., 2002). Together with some minerals (Na, K, and Cl), lactose contributes to the equilibrium of the blood–milk barrier, being the main osmotic regulator between the blood and alveolar lumen. In fact, lactose determines the amount of absorbed water in the alveoli, and thus, the volume of produced milk (Fox et al., 2015). As soon as lactose is synthesized by the Golgi, it is packed into secretory vesicles. Here, LP determines a strong osmotic pressure, because this disaccharide cannot pass through the vesicle membrane; water is required to get into the secretory vesicles and re-establish equilibrium.

The uptake of the precursor (glucose) from the circulatory system is regulated by facilitative glucose transporters, whose genetic expression also directly affects milk synthesis (Zhao, 2014). After translocation, operated both by glucose transporters 1 and 8, and by Na^+-dependent transport, glucose is partly absorbed into the Golgi of the epithelial cells and partly epimerized to uridine diphosphate (**UDP**)–glucose and then to UDP-galactose by the enzymatic actions of UDP-glucose-pyrophosphotylase-2 and phosphoglucomutase-1 (Figure 2). The same transporters carry UDP-galactose into the Golgi, where the lactose synthase, a heterodimer enzyme composed by α-LA and β-1,4-galactosyltransferase, catalyzes this chemical compound, releasing the UDP fragment. In particular, β-1,4-galactosyltransferase connects the carbon atom 1 of galactose and the carbon atom 4 of glucose. The role of α-LA is to increase the specificity of β-1,4-galactosyltransferase for glucose, so its concentration is usually highly correlated with the amount of lactose in milk (Fox et al., 2015). After formation, lactose-containing vesicles are released into the alveolar lumen from the apical membrane of the cell through facilitated transport (Zhao, 2014).

Lactose Forms

The glycosylic bond (1,4) connects the carbon atom 1 of galactose and the carbon atom 4 of glucose and, as for other carbohydrates, lactose might assume 2 anomeric forms: o-β-D-galactopyranosyl-(1,4)-α-D-glucopyranose, known as α-lactose, and o-β-D-galactopyranosyl-(1,4)-β-D-glucopyranose, known as β-lactose. At 20°C, total lactose is composed of 37.3% α-lactose and 62.7% β-lactose; the dynamic equilibrium between the 2 forms is influenced by factors such as total LP, temperature, pH, and the presence of co-solutes. The α-lactose increases its stability when associating with 1 molecule of water, defined as water of crystallization; for this

reason, it is usually referred as lactose monohydrate, and its molar weight is 360.31 g/mol (Fox et al., 2015). A transition between the 2 configurations occurs when a glucose monomer converts to an open aldeide form, with acid, base, or water acting as a catalyzing agent. As a result, anomeric acetal carbon of glucose can change its configuration from α to β, or vice versa. The 2 lactose isoforms differ in solubility, specific rotation, and sweetness. Such differences are crucial for technological treatments of lactose, such as spray-drying, crystallization, and downstream applications. Both α- and β-lactose exhibit the same nutritional profile (Fox et

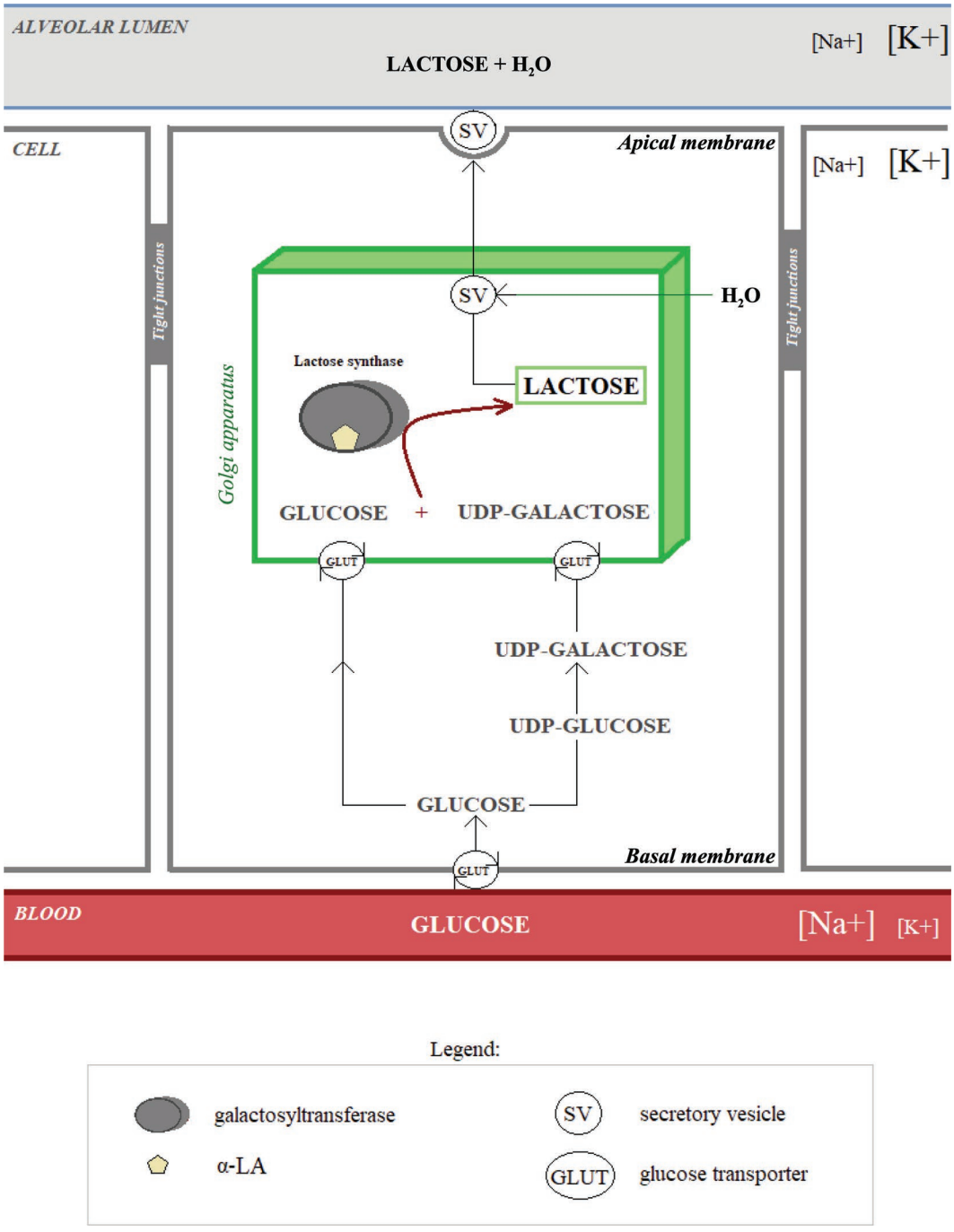

Figure 2. Schematic representation of the steps required for milk lactose synthesis in mammary epithelial cells. UDP = uridine diphosphate.

al., 2015), so for convenience, the ring tests coordinated by the International Committee for Animal Recording consider α-lactose to be the reference for laboratory comparison. The aldehydic form of lactose is present in milk in very low amounts and with a highly dynamic equilibrium; it tends to be immediately converted to the cyclic form. Nevertheless, the aldehyde group acts as a reducing agent and can interact with proteins through Maillard reactions.

HUMAN INTOLERANCE

Lactase enzyme, called β-galactosidase, hydrolyzes lactose into glucose and galactose in the digestive tract of mammal newborns. This enzyme is synthesized in the small intestine by microvilli, and in humans it is encoded by the lactase gene on chromosome 2 from 135.787 to 135.837 Mb (Domínguez-Jiménez and Fernández-Suárez, 2017; NCBI, 2018). Depending on population structure and genetic predisposition, the synthesis of this enzyme may decrease progressively with age, leading to difficulties in digesting milk and food containing lactose (Leonardi et al., 2012). This disorder, typically referred to as "lactose intolerance," is characterized by diarrhea and abdominal pain occurring immediately after the ingestion of lactose. In fact, as soon as lactose enters the intestinal environment, its concentration increases because of the absence of the lactase enzyme. This induces water to move from the blood into the intestinal tract to re-establish osmotic equilibrium, and it activates anomalous fermentations, with subsequent abdominal bloating and stomach pain.

Some differences exist between populations in toleration of lactose; for example, people from Nordic European countries and some African populations have a genetic predisposition for synthesizing lactase even in late age (Fox et al., 2015). Approximately 70% of the world population is at high risk of manifesting lactose intolerance, and prevalence has increased in the last decade, explaining why lactose-free products have gained importance in the marketplace (Domínguez-Jiménez and Fernández-Suárez, 2017). The most popular technological treatment for obtaining lactose-free milk is ultrafiltration (McCain et al., 2018), although it leads to an undesired removal of minerals from milk. To avoid the loss of important milk components, other methods have been developed to depress lactose through hydrolysis (McCain et al., 2018). For example, the addition of enzymes, specific molds, or yeasts (e.g., *Aspergillus* and *Kluyveromyces* spp.) during cheesemaking, milk heat treatment, or both allows for the separation of galactose and glucose, which are more digestible (Fox et al., 2015). The natural production of lactose-free milk in bovine species is biologically impossible, because lactose is the main milk osmole.

MILK LACTOSE: APPLICATIONS AND MARKET DEMAND

Industrial Uses

After cheesemaking, more than 90% of the lactose ends up in whey (Prazeres et al., 2012). Whey composition differs from that of milk, with average water, fat, and protein content of 93.9, 0.20, and 0.60%, respectively. Along with lactose, non-casein-related proteins and water-soluble vitamins are also found in the whey (Prazeres et al., 2012; Sturaro et al., 2014). The processing of 100 kg of whole milk into cheese leads on average to 85 kg of whey, which yields 5.36 kg of whey powder (CLAL, 2018). Therefore, the major international cheese manufacturers—the Netherlands, Germany, France, and Italy (CLAL, 2018)—produce significant amounts of whey, which was previously discarded as a byproduct of cheese factories and either dumped or fed to animals. Although alternative uses for whey are scarce, whey is still common in the liquid feeding system of Italian heavy pigs (up to 25% of ration; Martelli et al., 2002). With the purpose of improving the efficiency of the dairy industry, several studies have shown that whey is a source of exploitable high-nutrition and high-value compounds (Sturaro et al., 2014). Among the available methodologies, filtration is the most commonly adopted technology in the dairy industry for extracting lactose, because of its quality-to-cost ratio. Membrane separation was introduced in the first years of the 21st century, and it allows solid recovery and (purified) water-saving. Whey filtration is performed using different membranes at different retention efficiencies: ultrafiltration (<40% of lactose retention) and nanofiltration (>90% of lactose retention). The purity of crystalized solid lactose could be improved through reverse osmosis, concentration, and spray-drying (Prazeres et al., 2012). Depending on whether the recrystallization process is used in the last step, lactose powder is sold in either pharmaceutical and edible forms, coded as HS170211 and HS170219, respectively, in the Harmonized System nomenclature (CLAL, 2018). The pharmaceutical form is used as excipient for drug tablets, and edible lactose is commonly used as a base for confectionery and infant formula. The sweetness of lactose is less intense than that of common sugars (around 20% lower), making it an important ingredient for baked goods, ice creams, chocolate, and candies.

Whey permeate including lactose can be used as feed substrate for *Xanthomonas* bacteria in xanthan

gum plants (Fox et al., 2015; Niknezhad et al., 2015). Xanthan gum is mostly used by food factories for its physical properties and industrial applications (García-Ochoa et al., 2000; Murad et al., 2017). Pretreated hydrolyzed lactose is reported to be the most efficient and cheapest source of carbon for the production and excretion of this biopolymer by *Xanthomonas campestris* (Murad et al., 2017).

Market Demand

In January 2014, the price of lactose on the international market reached US$1,826/t, the highest historical value ever reached in the Global Dairy Trade auctions, but then declined to an average value of about US$800/t (Global Dairy Trade, 2018). In September 2018, the price was US$917/t (Global Dairy Trade, 2018), driven by high demand for powders from Asia, but this demand slowed with the Chinese market financial crisis.

The international standard for milk-powder production set an LP standard in the starting matrix, so in some countries, such as New Zealand, solid lactose has been imported for a long time from supplier countries (Lithuania, the Netherlands, Denmark, the United States, Germany, and Australia; CLAL, 2018) to reach the right starting milk composition (Sneddon et al., 2015) and to maximize milk-powder yield. Nowadays, for the same purpose, New Zealand uses lactose derived from the whey left after cheesemaking. Indeed, production and import/export of lactose is a business that involves several countries (Geary et al., 2010; CLAL, 2018). For instance, in Italy the export volumes and the market value of edible lactose increased from 2014 to 2017. In recent years, the market value for edible lactose has moved from €2.00 to €5.50/kg for global exports and from €1.20 to €6.08/kg for European Union exports (CLAL, 2018). In the global market, the export of lactose powders has shown a linear positive trend over the years, with the major exporters being Germany and the Netherlands. The exports of pharmaceutical and edible lactose in 2012 were 142,617 and 9,352 t, respectively, and in 2017 they increased to 189,994 and 12,429 t, respectively. Global demand for pharmaceutical lactose was evidently greater than that for edible lactose (CLAL, 2018), but trends in 2017 were both positive and increased by 9.6% (pharmaceutical) and 14.6% (edible) compared with 2016. It is also important to consider United States stocks of lactose, which could drive the global availability and market price of this product. According to Italy and to its whey surplus, both pharmaceutical and edible lactose powders are sold in the international market, with exports in 2017 of 29,777 and 112 t, respectively (CLAL, 2018); in particular, the Italian export of edible lactose in 2017 reached a very high peak (+350%) compared with previous years; the major international importers were Slovenia (39.0%), Australia (16.9%), and Romania (10.6%; CLAL, 2018). France imported one-third of the pharmaceutical lactose produced in Italy in 2017, and other importers included Spain, the Netherlands, Iran, and the Russian Federation (CLAL, 2018).

QUANTIFICATION OF LACTOSE AND VARIATION IN BOVINE MILK

According to ISO 22662:2007 (ISO, 2007), the reference method for lactose determination in raw, heat-treated, and dried milk is HPLC. However, with ISO 26462:2010 (ISO, 2010), the enzymatic method using difference in pH has been accepted for lactose quantification in milk samples. In the last 2 decades, introduction of the infrared spectroscopy technique in the routine analysis of milk has led to a revolution in dairy monitoring systems, allowing for monthly determination of LP in milk at the individual cow level (De Marchi et al., 2014). The predictions of LP from spectra are accepted for both scientific and economic purposes; indeed, a correlation of 0.996 in validation studies has been declared between measured and predicted LP (application note 5373 Rev. 3, MilkoScan 7RM/FT+/6000; Foss, Hillerød, Denmark). Some novel automatic and conventional milking systems are equipped with optic in-line measurement of milk composition, including LP and physical parameters (Lely Holdings, the Netherlands; Fullwood Ltd., United Kingdom). These technologies are becoming important tools, because their software can collect and store data and elaborate on information, reporting alerts for cows with potential problems, so that farmers can provide special treatment. In the case of LP, dramatic changes or specific patterns could be informative with respect to the cow's mammary gland health and energy balance; however, no studies have investigated LP changes and patterns in dairy cattle so far.

Milk LP shows variability, which depends on several factors (Fox et al., 2015). Studies discussing the effect of parity on LP are summarized in Table 1. Milk from first-calving cows has higher LP than milk from cows in later lactations; Haile-Mariam and Pryce (2017) and Costa et al. (2018) reported a gradual decrease in LP across parities in Australian dairy cattle and Italian Holsteins, respectively. Even if differences in LP are present among all parity orders, the major gap is between primiparous and multiparous animals (Løvendahl and Weibjerg, 2017; Costa et al., 2018). It is important

to consider that mastitis is usually more common in herds with a high percentage of multiparous cows, because primiparous cows tend to be less susceptible to udder inflammation; also, the milk of multiparous cows has generally higher SCC than the milk of primiparous cows (Harmon, 1994; Koeck et al., 2010). Because LP tends to decrease when clinical or subclinical udder inflammation is present and SCC increases, the LP gap between parities could be explained by the difference in milk SCC across cows of different lactations. A schematic representation of mechanisms relating LP, LY, and mastitis is depicted in Figure 3. In addition, a physiological (but not yet studied) mechanism could be that multiparous cows produce more milk, but with lower LP. This could suggest that both the osmotic function of lactose and the osmotic equilibrium between blood and milk could change in different parities. Finally, the cumulative effect of mastitis cases, repeated lactations, stage of lactation, and aging could affect mammary epithelium integrity and permeability, translating into gradual LP reduction during the productive life of a cow (Zhao, 2014; Herve et al., 2018). Lactose percentage does not show the usual lactation curve shape of fat and protein percentages; indeed, the lactation curve of LP is strictly related to that of milk yield, as already described in the section on the physiological mechanisms and pathways of lactose synthesis at the mammary level. Lactose is not affected by milk dilution through DIM, and it reaches the lowest values in late lactation, similar to milk yield and in an opposite trend to fat and protein percentages (Ptak et al., 2012; Penasa et al., 2016; Haile-Mariam and Pryce, 2017).

Effects of the dietary energy level on LP have also been investigated. Xue et al. (2011) reported significantly greater LP in the milk of cows fed a high dietary concentrate, and Ouweltjes et al. (2007) reported similar outcomes in a Dutch study; in particular, cows fed a high-energy diet had higher milk LP than cows fed a low-energy ration. Beerda et al. (2007) reported that LP significantly decreased by 15% in the milk of cows fed a low-caloric-density diet. Internationally, diet manipulation to increase LP is not economically justified or possible in the current era. Indeed, even major dairy powder producers and exporters such as New Zealand do not have feeding recommendations to increase milk LP (Sneddon et al., 2015).

MILK LACTOSE AND HEALTH TRAITS

Lactose as Biomarker of Metabolic Disorders

Glycemia and energy balance in cows are positively correlated with LP (Reist et al., 2002; Larsen and Moyes, 2015), especially in high-producing breeds (Lemosquet et al., 2009). Lemosquet et al. (2009) suggested that post-hepatic blood glucose availability could be an indirect key regulator of milk yield, making blood glucose directly responsible for LY. Hence, it is important to highlight the dependence of milk yield on LY and that the uptake of glucose from the blood to produce lactose is a metabolic priority in specialized dairy animals. In fact, udder requirements are subjected to homeorhesis in high-producing cows; that is, milk composition is unaltered even in case of breakdown of body reserves (Bauman and Currie, 1980; Zhao, 2014). For example, a sudden decrease of dietary energy level or increase in energy demand in high-yield cows could result in a negative energy balance and mobilization of fat reserves from tissue to blood, passing through the liver. Supporting this view, a negative phenotypic association (−0.17) between LP and milk BHB, one of the most common indicators of ketosis in dairy cows, has been reported by Larsen and Moyes (2015). Indeed, BHB levels can be measured in individual milk through keto tests or infrared milk analysis, and may help farmers identify cows in negative energy balance and ketosis. In fact, because LP is strictly dependent on blood circulating glucose, milk from (sub)ketotic cows tends to show lower LP and higher BHB than healthy animals, especially in early lactation. Evaluating the association between blood parameters and milk composition traits, Cant et al. (2002) reported a significant difference in LP between cows infused with 2 different solutions (glucose vs. saline), indirectly confirming the relation between LP and (sub)clinical ketosis. An average phenotypic correlation of −0.17 was found between LP and blood BHB in Norwegian Red cows (Belay et al., 2017). In addition, in a study in Fleckvieh cows,

Table 1. Summary of the literature dealing with a decrease of milk lactose percentage by increasing parity and SCC/SCS

Item	Reference
Parity	Miglior et al. (2006), Ptak et al. (2012), Malchiodi et al. (2014), Fox et al. (2015), Alessio et al. (2016), Haile-Mariam and Pryce (2017), Costa et al. (2018)
SCC/SCS	Pyörälä (2003), Bansal et al. (2005), Forsbäck et al. (2010), Gillon et al. (2010), Malek dos Reis et al. (2013), Moyes et al. (2014), Cinar et al. (2015), Fox et al. (2015), Kester et al. (2015), Nasr and El-Tarabany (2017), Costa et al. (2018)

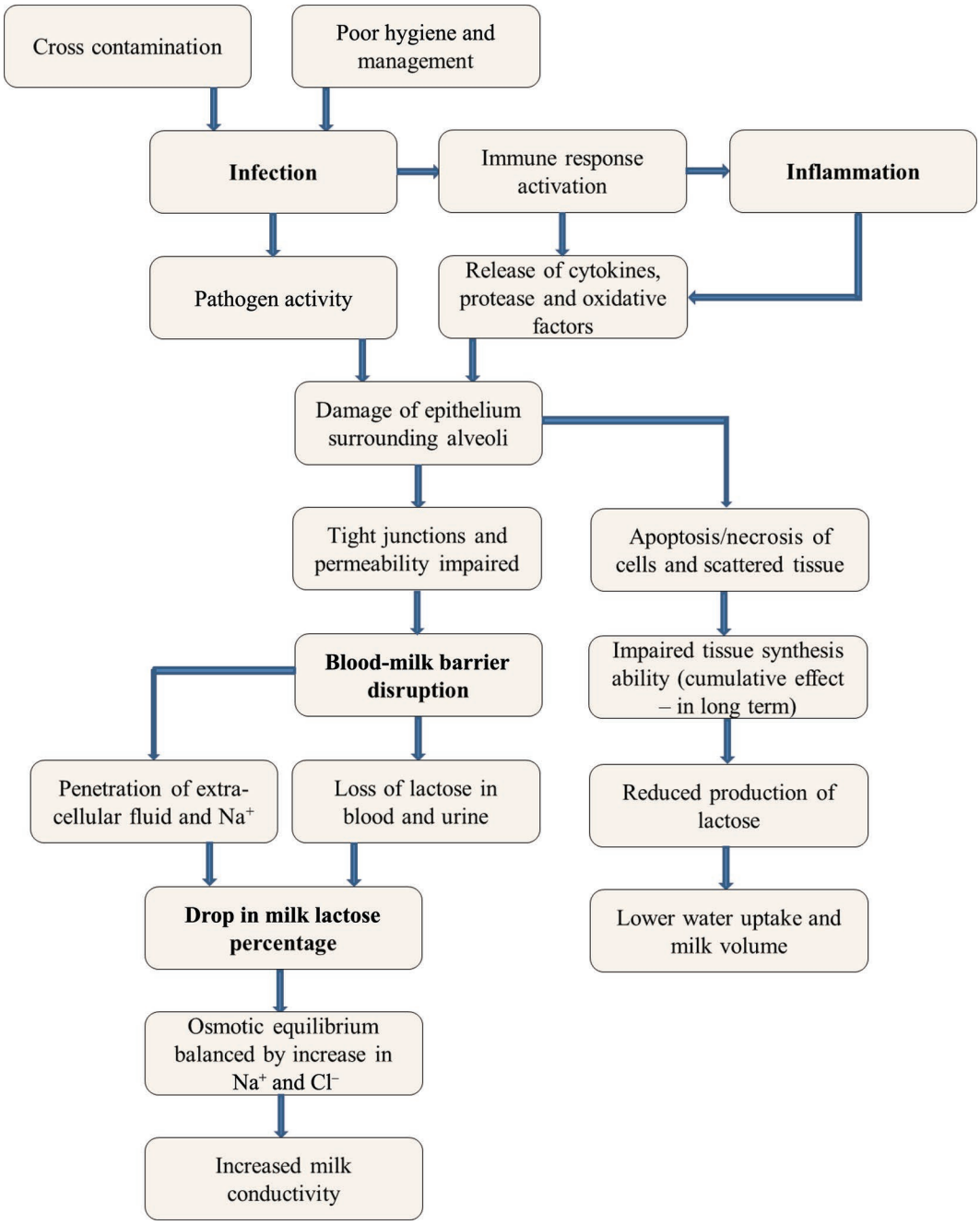

Figure 3. Diagram of the cause–effect relationships between lactose and mastitis in cows.

Ederer et al. (2014) found that ketosis was correlated with depressed LP (−0.15), increased fat percentage (0.21), and increased fat-to-lactose ratio (0.15) in early lactation, confirming that ketosis alters both fat percentage and LP in milk, even if with opposite effects. These results suggest that the relationship between milk lactose and gluconeogenesis in dairy cows should be further investigated, to detect and propose novel health indicators in milk.

Lactose and Mastitis

Mastitis is a disease that can occur throughout an entire lactation, with peaks in the first few months after calving. Studies of the effects of high SCC on milk LP are summarized in Table 1. Phenotypic correlations between LP and SCS range from −0.15 (Hossein-Zadeh and Ardalan, 2011) to −0.66 (Vilas Boas et al., 2017); in this sense, LP has been widely reported to be one of

the most informative traits for mastitis diagnosis, other than SCC and milk electrical conductivity (Geary et al., 2014; Fox et al., 2015; Vilas Boas et al., 2017). The reduction of LP in milk during mastitis (Figure 3) has 3 main causes: (1) LP synthesis is partly compromised because secretory cells are damaged by inflammation and infection; (2) a great, but still undefined, part of the lactose is lost in urine, because of a disruption of tight junctions and altered permeability of the basal membrane of the mammary cells that separates blood and milk; (3) mastitis pathogens use available milk lactose as a substrate, reducing LP and increasing lactic acid in milk. During mammary tissue inflammation, the osmotic balance is maintained by an increase of Na^+ and Cl^-; in particular, Na^+ derived from the highly Na^+-concentrated extracellular environment is the main ion responsible for the increase of the electrical conductivity and salty taste of milk (Figure 3). In addition, a formula to estimate the Koestler number (**Kn**) is reported in the literature, which relates milk Cl^- (%) and LP (Fox et al., 2015): Kn = [(milk Cl^-) × 100]/(milk LP). The Kn could be used to discriminate normal (Kn <2.00) and abnormal (Kn >3.00) milk. Finally, for the same reason, the electrical conductivity of milk is negatively related to LP (Fox et al., 2015; Vilas Boas et al., 2017; Ebrahimie et al., 2018). This background suggests that the complementary information from LP, SCC, and electrical conductivity can be used to provide an accurate diagnosis of mastitis at the individual level, and some authors have recently highlighted the potential of alternative or derived traits (or both) as predictors of udder inflammation. For instance, in the machine learning-based study of Ebrahimie et al. (2018), LP and electrical conductivity were the most reliable indicators of subclinical mastitis (together with SCC) and were able to recognize predictive patterns of subclinical mastitis in Holstein cows reared in New Zealand. Standard acceptable definitions of biological markers have been given by the Biomarker Definitions Working Group in Clinical Pharmacology and Therapeutics (Atkinson et al., 2001): "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." Therefore, considering the existing relations between LP and the traits above, lactose and perhaps its ratios with fat, protein, or both could be considered as potential biomarkers for early-lactation metabolic diseases, as reported by Ederer et al. (2014) in Fleckvieh cows. Nevertheless, further research is needed to validate LP as a reliable indicator. The adoption of precision farming technologies on a large scale will allow for the daily monitoring of individual milk and patterns of certain milk components that could be used to detect health disorders (de Haas, 2003) and prompt treatment.

GENETICS OF MILK LACTOSE

Dairy Species and Cattle Breeds

Considering the most common dairy species, average milk LP of 4.10, 4.70, and 4.90% have been reported in the literature for goats, cows, and sheep, respectively (Fox et al., 2015). Milk from buffalo shows LP similar to cow milk (4.50 to 5.20%), but with more variation across countries and breeding systems (El-Salam and El-Shibiny, 2011). Some studies (Malchiodi et al., 2014; Gottardo et al., 2017) have investigated the effect of cattle breed on milk composition, but there is no consensus on the effect of breed on LP.

Heritability and Repeatability

A summary of LP heritabilities for the first 3 lactations in dairy cows is presented in Figure 4. Lactose percentage usually shows higher heritability than milk yield and other milk solids (Gillon et al., 2010; Sneddon et al., 2015; Haile-Mariam and Pryce, 2017), with a moderate to high contribution of additive genetic variance to phenotypic variance. A heritability of nearly 0.40 is generally considered for LP in Holstein-Friesian cattle; however, there is no consensus on trends of heritability across parities. In fact, heritabilities of 0.478, 0.506, and 0.508 have been reported for Canadian Holsteins in parities 1, 2, and 3, respectively; on the other hand, Rzewuska and Strabel (2013) have reported heritabilities that were higher in first- (0.34) than in second- (0.28) and third-parity (0.26) Polish Holstein cows. Other studies (Haile-Mariam and Pryce, 2017; Satoła et al., 2017) did not detect differences in LP heritability across parity number (Figure 4). According to Haile-Mariam and Pryce (2017), LP heritability increased in the first 150 DIM and remained almost stable thereafter. Moreover, Belay et al. (2017) reported heritability of LP in the first half of lactation that was 0.406 from 11 to 30 DIM and 0.458 from 61 to 90 DIM. Genetic studies dealing with estimates of test-day records reported medium to high repeatability for LP (Table 2), suggesting that few observations within lactation are enough to capture the overall variability in LP. Estimates of heritability for LY range from 0.10 to 0.20. Due to the very high correlation of LY with milk yield, genetic parameters have been reported in a few countries so far, including New Zealand (Sneddon et al., 2015), Australia (Haile-Mariam and Pryce, 2017), and Italy (Tiezzi et al., 2013; Costa et al., 2018).

Table 2. Published estimates of heritability and repeatability (SE) of lactose percentage

Reference	Test days, no.	Breed	Model	Heritability	Repeatability
Miglior et al. (2007)	60,645	Holstein	Random regression	0.50	—
Stoop et al. (2007)[1]	5,581	Holstein	Linear	0.64 (0.10)	0.72 (0.01)
Loker et al. (2012)	86,331	Holstein	Random regression	0.52 (0.03)	—
Ptak et al. (2012)	48,859	Holstein	Random regression	0.24 (0.03)	—
Tiezzi et al. (2013)	63,470	Holstein	Linear	0.33	0.56
Ederer et al. (2014)	97,146[2]	Fleckvieh	Linear	0.32 (0.01)	—
Sneddon et al. (2015)	15,366	Mix	Linear	0.25 (0.04)	0.60 (0.01)
Belay et al. (2017)	717,915	Norwegian Red	Linear	0.43 (0.06)	—
Haile-Mariam and Pryce (2017)	724,325	Mix	Random regression	0.34	—
Satoła et al. (2017)	104,875	Holstein	Random regression	0.30	—
Visentin et al. (2017)	128,510	Mix	Random regression	0.36 (0.02)	0.49 (0.01)
Costa et al. (2018)	59,811	Holstein	Linear	0.43 (0.03)	0.63 (0.01)
Costa et al. (2019)	142,285[3]	Fleckvieh	Linear	0.57 (0.01)	0.62 (0.01)

[1]First-calving cows.

[2]Lactation records.

[3]Lactation records from the first 150 DIM.

Genetic Correlations with Traditional Milk Traits

Lactose percentage is not included in any breeding program for dairy cattle around the world, but it is routinely available as a part of normal herd testing and has been widely included in several genetic studies. However, it has been treated as an "accessory" and seldom discussed, likely due to its low and variable economic merit. Some genetic correlations involving LP exist in the literature, but further studies are required to improve our understanding of the genetic aspects of this trait and its relationship with other milk components. Milk yield and LP have been reported to be weakly genetically correlated (Miglior et al., 2007; Samoré et al., 2010; Sneddon et al., 2015; Visentin et al., 2017); in fact, LP is osmotically determined by the amount of water absorbed from the cell cytosol and the blood. Therefore, at least theoretically, LP is independent of

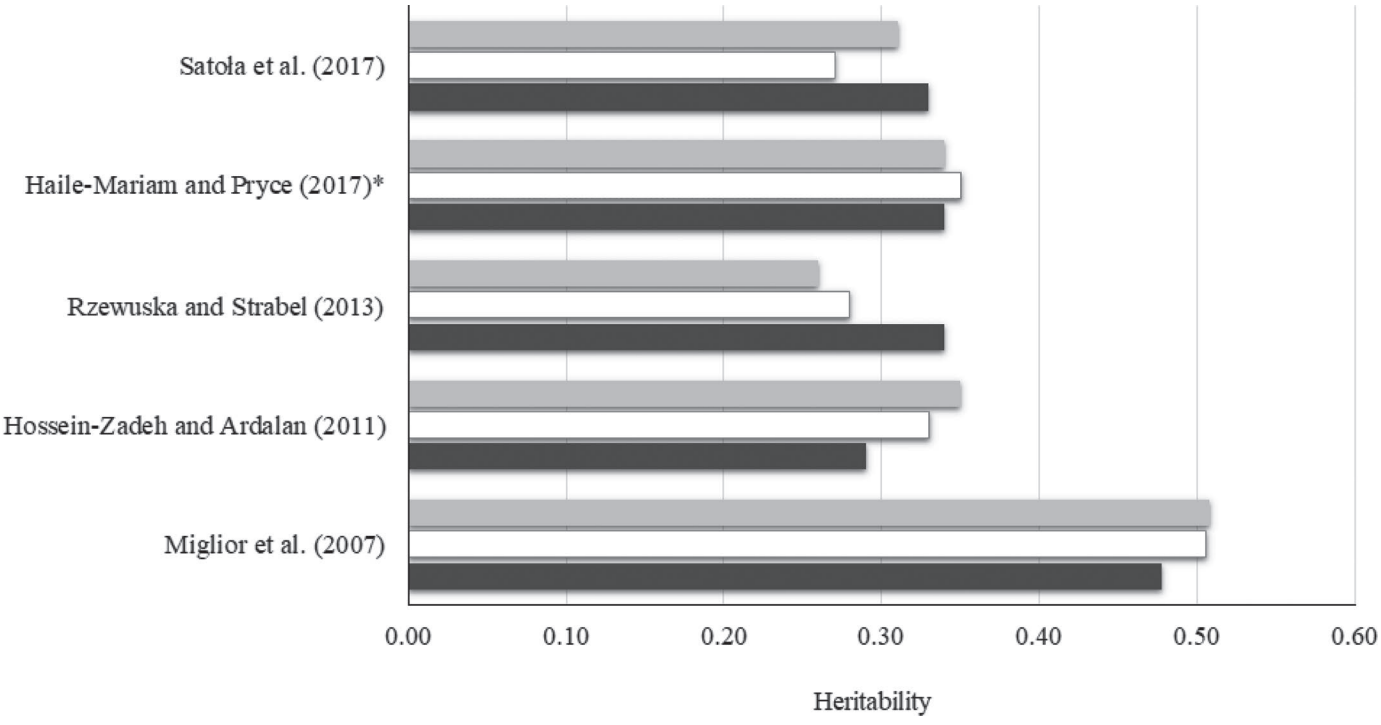

Figure 4. Estimates of heritability for lactose percentage in the milk of Holstein cows of different parities (parity 1 = black bars; parity 2 = white bars; parity 3 = gray bars). *Cows in Haile-Mariam and Pryce (2017) study were 75% Holstein, 15% Holstein × Jersey, and 10% Jersey.

LY, but the physiological pathways are not fully understood. Lactose yield, on the other hand, is strongly genetically associated with milk yield, with estimates close to 1 (Sneddon et al., 2012, 2015; Haile-Mariam and Pryce, 2017), and thus genetic correlations of LY or milk yield with other traits are similar (Sneddon et al., 2012, 2015). Genetic correlations of LP with fat and protein percentages are weak or close to zero (Miglior et al., 2007; Stoop et al., 2007; Visentin et al., 2017). However, using random regression models, some authors have reported variations in covariance estimates both within and across lactations (Haile-Mariam and Pryce, 2017; Satoła et al., 2017). For instance, Satoła et al. (2017) reported a trend for genetic correlations of LP with fat and protein percentages across DIM of first-parity cows that resembled the shape of the lactation curve for milk yield, with a peak in early lactation. Haile-Mariam and Pryce (2017) observed a shift from moderately positive (0.30) to moderately negative (−0.24) genetic correlation between LP and protein percentage moving from early to late lactation. Satoła et al. (2017) reported stronger genetic associations of LP with fat and protein percentages in first parities compared to later parities, whereas Haile-Mariam and Pryce (2017) found stronger correlations between LP and fat and protein percentage in third-parity cows compared to first-parity cows. Genetic relationships between casein (which plays a fundamental role in the cheesemaking process) and LP and LY have not been reported or discussed in the scientific literature so far. A moderate negative genetic correlation (−0.46) between LP and milk freezing point has been reported by Costa et al. (2018), and this result was somewhat expected, because milk freezing point, which is an indicator of milk dilution, is affected by the concentration of milk solids, the most abundant being lactose.

Genetic Correlations with Health Traits

Lactose has been already suggested as a potential health indicator in cows (Reist et al., 2002; Pyörälä, 2003; Bansal et al., 2005; Forsbäck et al., 2010; Gillon et al., 2010; Ederer et al., 2014; Haile-Mariam and Pryce, 2017). A collection of published genetic and phenotypic correlations between LP and SCS is presented in Table 3. Genetic correlations of −0.24 and −0.10 have been reported between LP and clinical mastitis in early lactation or across the whole lactation, respectively (Bastin et al., 2016), and Costa et al. (2019) reported a genetic correlation of −0.18 between LP and mastitis in the first 150 DIM of Fleckvieh cows. Traditional traits used for mastitis identification, such as SCS, are not always reliable across breeds or parity orders (Gillon et al., 2010). In fact, the correlation between mastitis and SCS is not always strong, with estimates from 0.30 to 0.70 (Mrode et al., 1998). All of these considerations stress SCS may not be sufficient to diagnose mastitis, because different pathogens affect milk SCS in different ways and magnitudes (dos Reis et al., 2013; Bobbo et al., 2017). Besides the association with mastitis and SCS, milk LP is also strictly related to cow energy balance and available blood glucose (see Biosynthesis Pathways, above). Moreover, the fat-to-lactose ratio in early lactation has been reported to be an indicator of cow energy balance, heritable (0.19), and genetically associated with clinical ketosis (−0.25; Ederer et al., 2014; Bastin et al., 2016). In addition, LP seems to be negatively related to BHB (blood and milk concentrations) and milk fat-to-protein ratio (Loker et al., 2012; Belay et al., 2017), usually referred to as markers of ketosis and negative energy balance. In fact, Belay et al. (2017) estimated negative genetic correlations between LP and blood BHB, with values of −0.234, −0.172, −0.159, and −0.154 from 11 to 30, 31 to 60, 61 to 90, and 91 to 120 DIM, respectively. In the same study, using a restricted data set, genetic correlations of LP and LY with ketosis were −0.043 and 0.161, respectively. Moreover, both LP and LY were genetically associated with ketosis in Fleckvieh breed, with estimates of −0.16 and 0.42, respectively (Costa et al., 2019). A positive association of LP with fertility in the subsequent lactation has been reported by Bastin et al. (2016), highlighting better fertility in cows yielding milk with higher LP. Both milk LP and fertility depend on cow energy balance (Bastin et al., 2016), meaning that their relationship is likely indirect. On the other hand, Costa et al. (2019) found that genetic correlations were close to zero between LP and some fertility disorders, namely retained placenta and ovarian cysts. In the last decade, several authors have coupled LP and milk urea as an indicator of metabolic health (Miglior et al., 2006, 2007; Loker et al., 2012; Satoła et al., 2017). These 2 traits are negatively associated, with an average genetic correlation of −0.15, and Miglior et al. (2007) reported that the genetic correlation was stronger in later-parity cows than in first-parity cows. Furthermore, Loker et al. (2012) reported a positive genetic correlation between LP and body condition score at 5 (0.27), 50 (0.31), and 150 (0.25) DIM, again suggesting that generally healthier cows produce milk with greater LP.

Finally, it should always be considered that LP has a moderate to high heritability, even across breeds and parities, whereas health traits do not (Egger-Danner et al., 2015; Pryce et al., 2016; Martin et al., 2018). On the other hand, LP has limited variability, which makes its use for genetic purposes difficult (Costa et al., 2018). Despite this, the availability of daily infor-

Table 3. Estimates of phenotypic and genetic correlations (SE) between lactose percentage and SCC reported in the literature

Reference	Test days, no.	Cows, no.	Breed	Phenotypic correlation	Genetic correlation
Stoop et al. (2007)	5,581	1,953	Holstein	−0.24 (0.02)	−0.44 (0.21)
Miglior et al. (2007)	60,645	5,022	Holstein	−0.23	−0.20
Gillon et al. (2010)	590,083	113,905	Mix	−0.38	−0.35
Hossein-Zadeh and Ardalan (2011)	458,408	57,301	Holstein	−0.15	−0.19
Sneddon et al. (2015)	15,366	4,378	Mix	−0.19 (0.01)	−0.07 (0.14)
Vilas Boas et al. (2017)	680	268	Gyr	−0.66	—
Visentin et al. (2017)	128,510	9,824	Mix	—	−0.28
Costa et al. (2018)	59,811	4,355	Holstein	−0.25 (0.01)	−0.22 (0.08)

mation on LP in future could be useful for detecting health disorders and for validation studies. In fact, except for some recent efforts in Austrian Fleckvieh cows (Costa et al., 2019), no other studies have attempted to properly validate LP or its ratio to other milk solids as an indicator of disease.

Literature on Lactose EBV

Correlations between EBV of LP (and LY) with EBV of traits under selection are very scarce. Miglior et al. (2007) and Haile-Mariam and Pryce (2017) estimated negative associations between EBV of LP and EBV of SCS (−0.164) and SCC (−0.150), respectively. A weak association between EBV of LP and EBV of milk yield was reported in both studies, with values of 0.101 in Canadian Holsteins (Miglior et al., 2007) and 0.06 in Australian dairy cattle (Haile-Mariam and Pryce, 2017). Conversely, a strong correlation has been reported between EBV of LY and EBV of milk yield, confirming the strong genetic relationship between these traits (Sneddon et al., 2015; Haile-Mariam and Pryce, 2017). Miglior et al. (2007) found favorable associations between EBV of LP and EBV of Canadian Lifetime Profit Index (0.139), median suspensory (0.112), mammary system (0.100), udder depth (0.128), and lactation persistency (0.329), which was defined as the expected milk yield at 280 DIM as a percentage of milk yield on 60 DIM in lactation. Although weaker, the correlation between EBV of LP and EBV of udder depth assessed by Haile-Mariam and Pryce (2017) was also positive (0.05); the same study reported favorable correlations of LP EBV with EBV of longevity (0.07) and fertility (0.08), indicating that on average the daughters of top bulls for LP tend to live longer and show better fertility than the average population. Moreover, the lifetime trend of LP and LY (e.g., maturity rate and persistency) could also add information and be related to traits of economic interest. The potential main critical points for the inclusion of LP in health indexes are: (1) the lack of deep knowledge on this feature; (2) the unknown effect of lactose on milk technological traits; and (3) the absence of genetic evaluation for milk lactose worldwide. Finally, the inclusion of LP in a selection index would require preliminary cost–benefit analysis according to specific dairy-market conditions.

Genome-Wide Association Studies

Genome-wide association studies for LP and LY are very scarce in the literature. To the best of our knowledge, only Wickramasinghe et al. (2011), Lopdell et al. (2017), and Wang and Bovenhuis (2018) have performed such studies for LP in dairy cattle in the United States, New Zealand, and the Netherlands, respectively. The main purpose of Wickramasinghe et al. (2011) was to evaluate candidate genes responsible for high-value oligosaccharides in milk; *B4GALT1* on chromosome 9, related to the transport of glucose, was significant for lactose biosynthesis. In particular, the expression of this gene was higher in the first part of lactation, corresponding to peak milk yield. These results confirmed the key role of lactose in determining milk volume and suggested that these 2 traits shared the same genetic evolution. Indeed, dietary requirements and feed intake in newborns are higher in the initial phase of lactation, and then progressively decrease as weaning age approaches. Through genome-wide association studies of LP and LY, Lopdell et al. (2017) identified significant regions coding for LP and LY on the genome related to transport mechanisms and osmoregulation of milk components. According to Lopdell et al. (2017) and Wang and Bovenhuis (2018), several regions code for LP and are spread over chromosomes 2, 3, 12, 16, 20, and 28. Conversely, significant variants for LY were found on chromosomes 6 and 14 only (Lopdell et al., 2017).

LACTOSE IN MILK PAYMENT SYSTEMS

Payment systems (**PS**) are constructed as a method of approximating the true value of milk on the basis of its components and are the main tool of communication with the farmer (Geary et al., 2010). Single-component PS are based on volume of milk, regardless of milk

quality and composition; multiple-component PS take the form of linear equations that usually include yields of fat and protein with a penalty for milk volume, or yields of fat, protein, and lactose with a penalty for milk volume. Volume charges are calculated as the ratio of the sum of all volume-related costs (i.e., unrelated to the quantity of the final product) to the volume of milk processed. Economic values in multiple-component PS are obtained by distributing the industry revenue plus any volume charges according to the ratio(s) of the marginal revenues for each component. Marginal revenues for milk components can be generated using different approaches; for instance, Geary et al. (2010) used the marginal rate of technical substitution to generate component values. This approach is similar to that suggested by Ladd and Dunn (1979) for estimating the value of milk components to a dairy manufacturer. Lactose has been considered a low-value milk component in the past, but the situation has changed in recent years as lactose has gained economic interest at international level. The implications of including lactose in New Zealand milk PS have been discussed by Sneddon et al. (2013), who stressed the need for proper genetic investigations simulating the inclusion of lactose into the payment formula, to estimate reliable economic merit. Sneddon et al. (2013) highlighted that LP and LY are very rarely included in milk price equations worldwide, but some PS put positive emphasis on solids-nonfat, indirectly accounting for lactose, whereas other PS put negative weight on milk volume, and therefore indirectly also on LY. However, updated information is unavailable; the last report dealing with a summary of existing PS of the International Dairy Federation (IDF, 2006) dates back to 2006. Finally, due to industrial secrecy, the milk payment equations of dairy producers and milk collectors are often not published. The following section aims to describe the PS of some large companies that include or somehow account for lactose in their PS.

New Zealand

The milk PS in New Zealand is known as "A + B − C," adopted by a prominent dairy company, the Fonterra Co-operative Group. The formula accounts for fat (A) and protein (B) and places a penalty on milk volume (C). One exception to "A + B − C" in New Zealand is the PS used by Synlait Milk Ltd., which also considers LY when paying suppliers. The system is called "F + P + L – V," where F, P, L, and V are fat, protein, lactose, and milk yields. In particular, the economic values used during the 2010–2011 season were NZ$4.24/kg of fat, NZ$10.34/kg of protein, NZ$1.84/kg of lactose and –NZ$0.0324/L of milk volume (2010 average: NZ$1 = US$0.72), derived from the model proposed by Garrick and Lopez-Villalobos (2000). Comparing these 2 PS from New Zealand, Holmes et al. (2007) demonstrated that the inclusion of LY reduced the values of fat and protein by 7 to 9%, with a value of LY that ranged from −NZ$0.416 to NZ$2.000/kg, depending on the product portfolio of the milk and breed examined.

The Netherlands

FrieslandCampina is the main company in the Netherlands; its member farmers receive a so-called guaranteed price that is based on the monthly trend in the published milk prices of some North European benchmark reference companies, regardless of the performance of FrieslandCampina. However, because the quality of delivered milk differs among farms, the final amount paid depends on the supplied kilograms of protein, fat, and lactose at a ratio of 10:5:1. Fewer deductions are contemplated for fixed costs and cooperative schemes and, when appropriate, some premiums. The premiums are related to full or partial outdoor grazing, the Foqus planet, and special milk flows (FrieslandCampina, 2018).

Ireland

The Irish dairy industry uses the "A + B − C" PS, similar to the Fonterra Co-operative Group of New Zealand. However, some processors have set a penalty when LP is below a certain threshold (e.g., Dairygold Co-operative Society Ltd., 2011). In particular, penalties of €0.10, €0.05, and €0.025/kg of milk are applied when LP is below 4.000%, between 4.001 and 4.100%, and between 4.101 and 4.200%, respectively. The reason behind this penalty is the role of LP as a farm-specific indicator of udder health and as a proxy for processing ability of milk (Glanbiaconnect, 2016).

United States

Some PS in the United States follow a hundredweight (cwt) of milk plus fat or protein component, fat plus solids-not-fat, fat only, total milk solids, or volume of milk. This PS is complicated by a classification system, in which each class has different economic values (Jesse and Cropp, 2004). In particular, class I includes milk used for beverage products (i.e., "white" whole, low-fat, and skim milk in all container sizes, chocolate, and other flavored milks, liquid buttermilk, and eggnog). Class II milk is used for soft manufactured products, such as ice cream and other frozen dairy desserts, cottage cheese, and creams such as sour cream, aerosol whipped cream, whipping cream, half and half cream, and coffee cream.

Class III milk is used for cream cheese and hard cheese, and class IV milk is used for butter and some dry milk products (Jesse and Cropp, 2004). Because the final value of the milk to the farmer is a composite of these classes, as well as of federal dairy product price-support programs such as the milk income loss contract (Chang and Mishra, 2011), determination of the value of independent milk components is more complicated than for the New Zealand dairy industry. The class III milk price (milk used for cheese production) is the basis for the majority of milk payments to dairy producers and it indirectly accounts for lactose, because it is based on the value of solids, with different prices for milk protein, milk fat, and other solids (lactose and minerals). In November 2017, protein, fat, and other solids were valued at US$8.00, 3.59, and 0.88/kg, respectively. Dairy producers were paid on the basis of the amount of these 3 components; moreover, the class III price could be considered as an index that represents the value of milk with 3.00% protein, 3.50% fat, and 5.70% other solids (Geuss, 2013).

Canada

Dairy farmers of Ontario are paid according to butterfat, protein, and other solids supplied with their raw milk; therefore, this system indirectly accounts for lactose. The values for fat, protein, and other solids (lactose + minerals) used in May 2018 were CA$10.63, 6.39, and 1.36 (2018 average: CA$1 = US$0.7786), respectively (Dairy Farmers of Ontario, 2018).

CONCLUSIONS AND FUTURE CHALLENGES

So far, any genetic selection scheme for production that includes udder health and metabolic diseases accounts for LY or LP in dairy cattle worldwide (Interbull, 2018). Considering that both phenotypic and genetic correlations of LY with milk yield are close to unity, an indirect selection for LY already exists in these indexes, putting both positive and negative emphasis on milk yield. On the other hand, LP is moderately to highly heritable and genetically correlated with 2 important health traits, namely mastitis and ketosis. Nowadays, there is concern that selection based only on SCC/SCS will lead to a progressive loss of immune ability and immunological response to infections in subsequent generations (Rainard et al., 2018). Although this idea is still under debate and there is no consensus yet (Rainard et al., 2018), LP could be a potential additional trait for inclusion in udder health indexes along with SCC/SCS. Breeding for LP makes sense in this perspective. As reported by Martin et al. (2018), the addition of new traits such as LP in selection indexes will improve the EBV accuracy and allow for faster genetic improvement of traits of interest, such as mastitis resistance, udder health, and energy balance in future generations of dairy cows. However, reliable estimations of proper weights and economic values through population-level simulations are required. Economic values for LP and LY should be developed, taking into account specific objectives, dairy outputs, processing costs, and market prices, adapting and updating the model proposed by Garrick and Lopez-Villalobos (2000) in the desired directions. In New Zealand, Sneddon et al. (2016) estimated the correlated responses in LY, LP, and protein-to-protein-plus-lactose ratio following selection for breeding worth, breeding worth plus LY, breeding worth plus LP, and breeding worth plus protein-to-protein-plus-lactose ratio. The reason for these selection indexes was to evaluate whether cows might produce milk with the right protein-to-protein-plus-lactose ratio: that is a more suitable milk composition for the production of whole milk powder. Those authors concluded that the New Zealand dairy industry could reduce the import of foreign lactose by 6 to 11% per ton of whole milk powder by including lactose in the breeding objective, if compared with selection on solely breeding worth. However, before a new trait is included in any selection index for specific breeding goals, an evaluation of the potential pleiotropic effects is essential; therefore, further phenotypic and genetic investigations of LP and LY are required. In the era of big data, modern hi-tech milking systems can provide daily information about milk from individual cows; this would allow for a better understanding of the phenotypic and genetic "behavior" of milk lactose within and across lactations.

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Aims of the thesis

The overall objective of this thesis was to investigate lactose at genetic level in dairy cattle. The specific aims were:

i) to identify sources of variation of LP and LY;

ii) to assess genetic parameters of LP and LY;

iii) to evaluate phenotypic and genetic associations of LP and LY with milk yield, fat and protein percentage, SCS, freezing point, milk coagulation properties, and minerals content;

iv) to estimate genetic correlations of LP and LY with mastitis and other common health disorders of cows;

v) to identify genomic regions coding for LP and LY and search for regions in common with SCS and/or mastitis.

Chapter I

Heritability and repeatability of milk lactose and its relationships with traditional milk traits, somatic cell score and freezing point in Holstein cows

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Heritability and repeatability of milk lactose and its relationships with traditional milk traits, somatic cell score and freezing point in Holstein cows

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Lactose percentage (LP) in milk is currently determined in most herd-testing schemes, and globally, it is usually routinely recorded in the framework of the official milk recording procedures. However, few studies have investigated the phenotypic and genetic variability of this component. Data used in the present paper consisted of 59 811 test-day records from 4355 Holstein cows in 266 herds. Heritabilities of LP and lactose yield (LY) were estimated through single-trait repeatability animal models, whereas genetic and phenotypic correlations of LP and LY with milk composition and production traits, somatic cell score and milk freezing point were estimated using bivariate models. Fixed effects included in the analyses were herd-test-date, season of calving, parity, stage of lactation and the interaction between parity and stage of lactation. Random effects were animal additive genetic, within and across lactation permanent environment and the residual. Lactation curves of LP and LY increased from parturition to the peak of lactation and decreased thereafter, mirroring the typical curve of milk yield. Lactose percentage was greater in first- than later-parity cows. Heritabilities of LP and LY were 0.43 ± 0.03 and 0.14 ± 0.02, respectively, and LP and protein percentage were the most repeatable traits. Genetic correlations (r_a) of LP with somatic cell score, LY and milk freezing point were −0.22 ± 0.08, 0.28 ± 0.08 and −0.46 ± 0.05, respectively. Genetic relationships of LY with milk yield ($r_a = 0.97 \pm 0.00$), fat percentage ($r_a = -0.71 \pm 0.06$), protein percentage ($r_a = -0.57 \pm 0.06$) and protein yield ($r_a = 0.64 \pm 0.06$) were moderate to strong. Results suggest that milk LP could be considered in breeding strategies to accelerate the gain of correlated low heritable traits. Further research is needed to evaluate the feasibility of including LP in the selection index of Italian Holstein population to address country-specific needs and market demands.

Keywords: bovine, lactose, milk composition, genetic parameter, correlation

Implications

The present study quantified the phenotypic and genetic characteristics of bovine milk lactose. Lactose is the major sugar of mammals' milk and in solid form it is an important ingredient for different food and products. In addition, phenotypic studies reported that it can be used as biomarker for identification of cow udder inflammation. As literature on genetic aspects of bovine milk lactose is scarce, the parameters estimated in the present study are a contribution to increase the knowledge on the genetic background of milk lactose, making it exploitable for breeding purposes.

Introduction

Lactose is a disaccharide composed of glucose and galactose and it is the major carbohydrate in mammals' milk. Cow milk contains around 5% of lactose, which represents about 40% of total solids (Fox *et al.*, 2015). The Golgi apparatus is the organelle of the mammary gland cell where the synthesis of lactose takes place, starting from blood glucose. The amount of synthesized lactose is the major regulator of milk volume; in fact, due to different osmotic pressures between cell lumen and Golgi vesicles, water is transported into the Golgi apparatus to stabilize the osmotic equilibrium. This mechanism explains why lactose percentage (LP) is not affected by milk dilution, when compared with other milk solids whose concentration is affected by the volume of milk and drops at the peak of lactation (Fox *et al.*, 2015). The reference analysis of milk LP, typically expressed as monohydrate form, is the HPLC (ISO 22662, 2007). Infrared

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spectroscopy is routinely adopted to determine LP (ISO 9622, 2013), as the correlation between measured and predicted LP in validation is 0.996 (FOSS, Hillerød, Denmark).

Lactose plays a central role in milk powder production, being often the limiting milk solid. Considering that international standards require specific final composition of milk powders, some countries such as New Zealand have to purchase extra lactose from the international market in order to reach the proper composition of milk powder and exploit the amounts of fat and protein contained in the liquid milk or, alternatively, remove the excesses of fat and protein (World Health Organization and Food and Agriculture Organization of the United Nations, 2011; Sneddon *et al.*, 2016). In Italy, the production and exportation of solid lactose has increased since 2014, as a consequence of rising cheese and whey production (CLAL, 2017).

Lactose percentage is influenced by several sources of variation, mainly parity number, stage of lactation, udder health status and individual animal (Henao-Velásquez *et al.*, 2014; Fox *et al.*, 2015; Alessio *et al.*, 2016). Heritability of LP ranges from 0.25 to 0.51 (Miglior *et al.*, 2007; Gillon *et al.*, 2010; Haile-Mariam and Pryce, 2017). Because of the central role of lactose in regulating milk volume, phenotypic and genetic correlations between lactose yield (LY) and milk yield (MY) are close to unity (Sneddon *et al.*, 2015), and thus their lactation curves have the same pattern (Henao-Velásquez *et al.*, 2014). Likewise, the lactation curve of LP resembles those of MY and LY, but shows greater persistency and smaller variation across lactation (Miglior *et al.*, 2006; Leitner *et al.*, 2011). Negative correlation exists between milk LP and somatic cell score (SCS), meaning that lactose could be an indicator of udder health. Phenotypic and genetic correlations between LP and SCS range from −0.66 to −0.19 (Sneddon *et al.*, 2015; Vilas Boas *et al.*, 2017) and −0.35 to −0.07 (Gillon *et al.*, 2010; Sneddon *et al.*, 2015), respectively, depending on cow breed and statistical model. The drop of LP in the presence of high SCS is due to the increased permeability of basal membrane of cells during mammary tissue inflammation; however, the mechanisms behind this relation are not totally understood yet (Fox *et al.*, 2015). Finally, some reports have estimated negative phenotypic correlations between LP and milk freezing point (FRP), suggesting that high LP leads to lower (i.e. more desirable) milk FRP (Bjerg *et al.*, 2005; Kedzierska-Matysek *et al.*, 2011; Costa *et al.*, 2017).

The scientific community has only marginally investigated lactose as a feature of potential interest for the dairy industry, and there is a paucity of information on phenotypic and genetic aspects of this trait so far. Furthermore, Løvendahl and Weisbjerg (2017) have stressed the lack of genetic correlations between LP and other milk traits computed using big data sets. Gaining knowledge on the genetics of milk lactose could be useful to address breeding strategies that consider this compound, for example, as indicator of udder health of dairy cows. Hence, the aims of the present paper were to characterize phenotypic aspects of LP and LY, and to estimate their genetic parameters in Italian Holstein cows.

Material and methods

A total of 293 575 test-day records from 16 523 Holstein cows in 1097 herds were collected from January 2011 to December 2014 in the Province of Bolzano (North of Italy) during monthly milk recording. Milk yield (kg/day) for each test-day record was available. Lactose percentage, fat percentage (FP), protein percentage (PP) and FRP were determined with mid-IR spectroscopy using a MilkoScan™ FT6000 (Foss Electric A/S, Hillerød, Denmark), and somatic cell count (SCC, cells/μl) was assessed through Fossomatic™ FC (Foss Electric A/S). The milk analyses were performed in the laboratory of the South Tyrolean Dairy Association (Bolzano, Italy). Lactose yield, fat yield (FY) and protein yield (PY) expressed in kg/day were derived by multiplying the respective percentages by MY.

Cows were required to have known sire and dam, to be in parity 1 to 5 and between 6 and 480 days in milk. Based on the frequency distribution of age at calving within parity, records of animals outside the following ranges of age (months) were removed: 20 to 40 for first-parity, 32 to 58 for second-parity, 44 to 76 for third-parity, 56 to 94 for fourth-parity and 68 to 112 for fifth-parity cows. According to International Committee for Animal Recording (ICAR, 2016) guidelines, test-day records of LP, FP and PP outside the ranges 4.0% to 5.5%, 2.0% to 6.0% and 2.5% to 4.5%, respectively, were discarded from the data set. Somatic cell count was required to be between 1000 and 10 000 000 cells/ml, MY between mean ±3 SD and FRP between mean ±2 SD. To achieve normality and homogeneity of variance, SCC was converted to SCS using the formula $SCS = 3 + \log_2(SCC/100)$. A minimum of three records per cow within lactation and a minimum of three cows per herd-test-date (HTD) were required for subsequent statistical analyses. This restriction was established considering that the average herd size in Bolzano province is around 15 lactating cows and thus setting the minimum number of cows per HTD to a higher threshold than three would have resulted in an excessive loss of test-day records. The final data set consisted of 150 633 records from 10 893 cows in 664 herds.

Statistical analysis

The software adopted for all analyses was ASReml 4.1 (Gilmour *et al.*, 2015). A phenotypic ANOVA of milk production and composition traits, SCS and FRP was performed using the following linear model:

$$Y_{ijklm} = \mu + \text{htd}_i + \text{parity}_j + \text{stage}_k + \text{season}_l + (\text{parity} \times \text{stage})_{jk} + \text{cow}_m + e_{ijklm}$$

where Y_{ijklm} is the dependent variable (MY, FY, PY, LY, FP, PP, LP, FRP or SCS); μ the overall intercept of the model; htd_i the fixed effect of the ith HTD ($i = 1$ to 18 402); parity_j the fixed effect of the jth parity number of the cow ($j = 1, 2, 3, 4, 5$); stage_k the fixed effect of the kth class of stage of lactation ($k = 6$ to 30, 31 to 60, 61 to 90, … , 301 to 330, 331 to 390, >390 days); season_l the fixed effect of the lth season of calving [(l = winter (December to February), spring (March to

May), summer (June to August) and autumn (September to November)]; (parity × stage)$_{jk}$ the fixed interaction effect between parity and stage of lactation; cow$_m$ the random effect of the mth cow ($m = 1$ to 10 893) ~ $N(0, \sigma^2_{cow})$; and e_{ijklm} the random residual ~ $N(0, \sigma^2_e)$.

In order to evaluate the impact of different levels of LP on milk production and composition traits (except for LY and LP), SCS and FRP, a second ANOVA was performed by adding the fixed effect of classes of LP to the previous model. Three classes were created according to mean ± 1 SD (class 1: LP < 4.59%; class 2: 4.59% ⩽ LP ⩽ 4.93%; class 3: LP > 4.93%) and LP averaged 4.47 ± 0.11%, 4.77 ± 0.09% and 5.01 ± 0.07% in class 1, 2 and 3, respectively. Pair-wise comparisons of means were performed for the effect of classes of LP using a t-test ($P < 0.05$).

To reduce computational time, genetic analysis was carried out on a randomly selected subset of herds that included 40% ($n = 266$) of the total number of herds in the edited data set ($n = 664$). The subset consisted of 59 811 records from 4355 cows and 7530 HTD. Means and variation of all traits and the frequency of observations within each parity in this subset (34%, 28%, 19%, 12% and 7% for parity 1, 2, 3, 4 and 5, respectively) reflected those of the whole data set. A six-generation pedigree was provided by the Italian Holstein Association (ANAFI, Cremona, Italy) and included 17 092 animals, that is all cows with records and their ancestors. Univariate repeatability animal models were used to estimate variance components for milk production and composition traits, SCS and FRP, and bivariate repeatability animal models were used to compute covariances between the traits. The general form of the univariate repeatability animal model, in matrix notation, was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Ww} + \mathbf{Sc} + \mathbf{e}$$

where **y** is the vector of phenotypic observations of the dependent variable (MY, LY, FY, PY, LP, FP, PP, SCS or FRP); **b** the vector of fixed effects (HTD, parity, stage of lactation, calving season, interaction between parity and stage of lactation); **a** the vector of random additive genetic effects; **w** the vector of random within-lactation permanent environmental effects; **c** the vector of random across-lactation permanent environmental effects; **e** the vector of random residuals; and **X**, **Z**, **W** and **S** are incidence matrices relating the corresponding effects to the dependent variable. The following expectations (E) of the variables were assumed: $E(\mathbf{y}) = \mathbf{Xb}$, $E(\mathbf{a}) = \mathbf{0}$, $E(\mathbf{w}) = \mathbf{0}$, $E(\mathbf{c}) = \mathbf{0}$ and $E(\mathbf{e}) = \mathbf{0}$. The variances of random effects were assumed as follows: $var(\mathbf{a}) = \mathbf{A}\sigma^2_a$, $var(\mathbf{w}) = \mathbf{I_1}\sigma^2_w$, $var(\mathbf{c}) = \mathbf{I_2}\sigma^2_c$ and $var(\mathbf{e}) = \mathbf{I_3}\sigma^2_e$, where σ^2_a is the additive genetic variance, σ^2_w the within-lactation permanent environmental variance, σ^2_c the across-lactation permanent environmental variance, σ^2_e the random residual variance, **A** the numerator relationship matrix between all animals considered in the data set, $\mathbf{I_1}$ is an identity matrix of order equal to the number of lactations in the data set; $\mathbf{I_2}$ is an identity matrix of order equal to the number of cows in the data set; and $\mathbf{I_3}$ is an identity matrix of order equal to the number of records.

The bivariate repeatability animal model for any pair of two traits could be generally represented as:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} + \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \begin{bmatrix} w_1 \\ w_2 \end{bmatrix} + \begin{bmatrix} S_1 & 0 \\ 0 & S_2 \end{bmatrix} \begin{bmatrix} c_1 \\ c_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

The following assumptions were considered: $E(y_1) = X_1 b_1$, $E(y_2) = X_2 b_2$, $E(a_i) = 0$, $E(w_i) = 0$, $E(c_i) = 0$ and $E(e_i) = 0$. The (co)variance structure of the random effects was assumed as follow:

$$\text{Var}\begin{bmatrix} a_1 \\ a_2 \\ w_1 \\ w_2 \\ c_1 \\ c_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} A\sigma^2_{a_1} & A\sigma_{a_{12}} & 0 & 0 & 0 & 0 & 0 & 0 \\ A\sigma_{a_{12}} & A\sigma^2_{a_2} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & I_1\sigma^2_{w_1} & I_1\sigma_{w_{12}} & 0 & 0 & 0 & 0 \\ 0 & 0 & I_1\sigma_{w_{12}} & I_1\sigma^2_{w_2} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & I_2\sigma^2_{c_1} & I_2\sigma_{c_{12}} & 0 & 0 \\ 0 & 0 & 0 & 0 & I_2\sigma_{c_{12}} & I_2\sigma^2_{c_2} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & I_3\sigma^2_{e_1} & I_3\sigma_{e_{12}} \\ 0 & 0 & 0 & 0 & 0 & 0 & I_3\sigma_{e_{12}} & I_3\sigma^2_{e_2} \end{bmatrix}$$

where σ_{a12}, σ_{w12}, σ_{c12} and σ_{e12} are genetic, within-lactation permanent environmental, across-lactation permanent environmental and residual covariances between traits 1 and 2, respectively. Heritability (h^2) and repeatability (t) of a trait were calculated as:

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_w + \sigma^2_c + \sigma^2_e}, t = \frac{\sigma^2_a + \sigma^2_w + \sigma^2_c}{\sigma^2_a + \sigma^2_w + \sigma^2_c + \sigma^2_e}$$

Genetic (r_a) and phenotypic (r_p) correlations were calculated as:

$$r_a = \frac{\sigma_{a_{12}}}{\sqrt{\sigma^2_{a_1} \times \sigma^2_{a_2}}}, r_p = \frac{\sigma_{p_{12}}}{\sqrt{\sigma^2_{p_1} \times \sigma^2_{p_2}}}$$

where σ^2_p is the phenotypic variance for any trait calculated as $\sigma^2_p = \sigma^2_a + \sigma^2_w + \sigma^2_c + \sigma^2_e$, and σ_{p12} the phenotypic covariance between traits 1 and 2, calculated as $\sigma_{p12} = \sigma_{a12} + \sigma_{w12} + \sigma_{c12} + \sigma_{e12}$.

Results

Descriptive statistics and analysis of variance

Descriptive statistics for the edited data set ($n = 150\,633$) are reported in Table 1. Lactose percentage averaged 4.76% with a CV of 3.57%. Lactose yield had a CV of 29.01%, close to the CV of MY (28.03%), FY (29.09%) and PY (25.00%). The greatest CV was obtained for SCS (60.82%) and the lowest for FRP (1.33%).

Fixed effects of HTD, parity, stage of lactation, season of calving and the interaction between parity and stage of lactation were significant ($P < 0.001$) in explaining the variation of milk production and composition traits, SCS and FRP. Least squares means of LP for parity are depicted in Figure 1; significant differences ($P < 0.05$) were observed

between cows in first- (4.82 ± 0.002%) and cows in third- (4.70 ± 0.002%), fourth- (4.68 ± 0.002%) and fifth-lactation (4.66 ± 0.003%). Parity order-specific lactation curves of LP, LY and MY (Figure 2) highlighted the greater persistency of LP and MY in younger compared with older animals, which led to greater LY in first-parity cows at the end of lactation. The fixed effect of classes of LP added to the second ANOVA was significant ($P < 0.001$) for MY, FP, PP, FY, PY, FRP and SCS. In particular, MY increased from 25.58 ± 0.05 to 26.94 ± 0.05 kg/day moving from low to high LP class, whereas FRP and SCS decreased from −0.519 ± 0.00°C to −0.530 ± 0.00°C and from 3.88 ± 0.02 units to 2.53 ± 0.02° units, respectively (Figure 3).

Genetic parameters

Estimates of variance components, heritability and repeatability for each trait are presented in Table 2. Lactose percentage had the highest heritability (0.43 ± 0.03) among the studied traits. Heritabilities were low for LY (0.14 ± 0.02), FRP (0.12 ± 0.01) and SCS (0.10 ± 0.02), and moderate for FP (0.35 ± 0.02) and PP (0.40 ± 0.03). Lactose percentage, PP, MY and LY were highly repeatable, with values between 0.59 ± 0.01 (LY) and 0.64 ± 0.01 (PP). Repeatabilities of the other traits ranged from 0.29 ± 0.01 (FRP) to 0.54 ± 0.01 (PY).

Table 1 *Descriptive statistics of quality and production traits of bovine milk (*n = *150 633)*

Traits	Mean	SD	Minimum	Maximum	CV (%)
Composition (%)					
Lactose	4.76	0.17	4.00	5.46	3.57
Fat	4.03	0.65	2.00	6.00	16.13
Protein	3.38	0.38	2.50	4.50	11.24
Production (kg/day)					
Milk	27.58	7.73	3.10	51.50	28.03
Lactose	1.31	0.38	0.13	2.68	29.01
Fat	1.10	0.32	0.08	3.05	29.09
Protein	0.92	0.23	0.11	2.17	25.00
Freezing point (°C)	− 0.525	0.007	− 0.545	− 0.505	1.33
SCS (units)	2.91	1.77	− 3.64	9.64	60.82

SCS = somatic cell score.

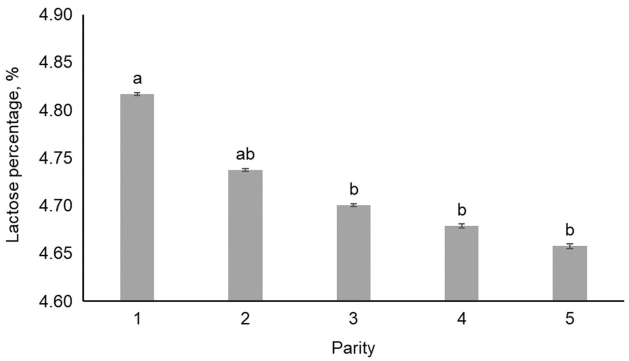

Figure 1 Least squares means (with SE) of milk lactose percentage across parities of dairy cows. [a,b]Means with different letters are significantly different ($P < 0.05$).

Phenotypically, LP was moderately correlated with FRP (−0.53 ± 0.01), SCS (−0.25 ± 0.01) and LY (0.23 ± 0.01), and it was uncorrelated with other production and quality traits (Table 3). Milk FRP was negatively associated with FP (−0.15 ± 0.01) and PP (−0.25 ± 0.01), and uncorrelated with LY, FY and PY. Somatic cell score exhibited correlations of −0.11 ± 0.01 with MY, −0.15 ± 0.01 with LY and close to 0 with the other features. Phenotypic correlations of LY with milk composition and production traits were generally stronger than the correlations involving LP, except for the associations between LY and FRP (−0.01 ± 0.01), and LY and SCS (−0.15 ± 0.01).

From a genetic point of view, LP correlated with LY (0.28 ± 0.08), SCS (−0.22 ± 0.08) and FRP (−0.46 ± 0.05),

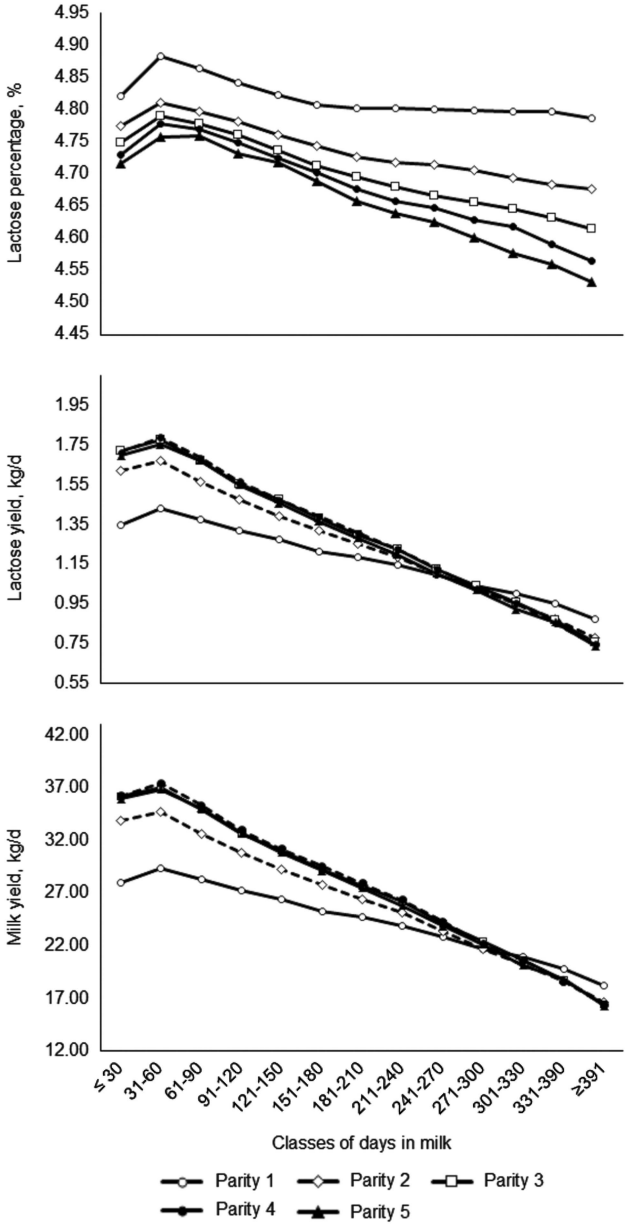

Figure 2 Least squares means of lactose percentage (SE from 0.00 to 0.01), lactose yield (SE from 0.00 to 0.01) and milk yield (SE from 0.07 to 0.22) of dairy cows for the interaction effect between classes of days in milk and parity.

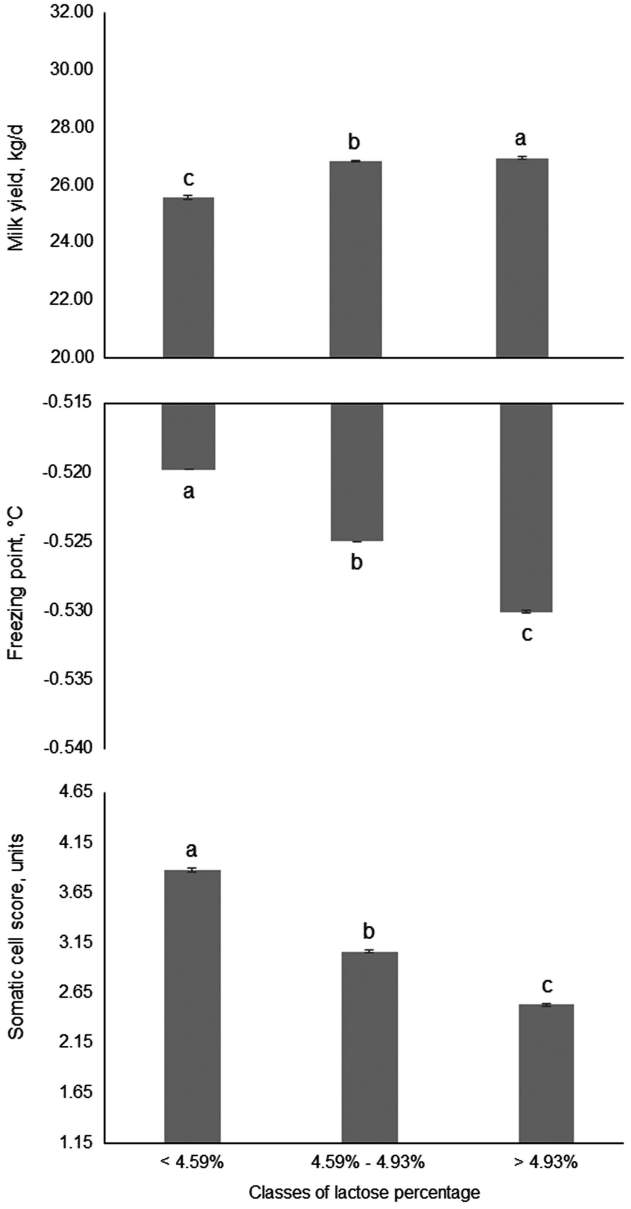

Figure 3 Least squares means (with SE) of cow milk yield, freezing point and somatic cell score across classes of lactose percentage. [a,b,c]Means with different letters are significantly different ($P < 0.05$).

and did not associate with FP, PP, MY, FY and PY (Table 3). The strongest associations involving LY were estimated with MY (0.97 ± 0.00), PY (0.64 ± 0.06), FP (−0.71 ± 0.06) and PP (−0.57 ± 0.06).

Discussion

Descriptive statistics

Milk composition was in accordance with results reported by Tiezzi *et al.* (2013), Penasa *et al.* (2015) and Toffanin *et al.* (2015) in Holstein cows. Lactose yield, FY and PY were slightly lower than mean values reported by Tiezzi *et al.* (2013). Overall, LP was comparable with the literature, but greater than the averages reported by Miglior *et al.* (2006) for Canadian Holsteins (4.58%), Glantz *et al.* (2012) for Swedish Holsteins (4.38%), Alessio *et al.* (2016) for Holstein and Jersey breeds (4.47%) and Petrini *et al.* (2016) for Holstein cows reared under tropical conditions (4.60%). Conversely, greater values were reported by Haile-Mariam and Pryce (2017) using data from Australian Holstein and Jersey herds; in particular, in their study, LP averaged 5.03%, 4.97% and 4.94% in first-, second- and third-lactation cows, respectively. Similarly, Sneddon *et al.* (2015) reported higher mean LP (5.12%) in a multi-breed study conducted in New Zealand. Indeed, this was mainly due to the feeding system based on pasture and seasonal calving. Lactose percentage of Australian and New Zealand milk is generally greater than that of European and American milk (Haile-Mariam and Pryce, 2017). The low CV of LP was expected as it is the direct consequence of the physiological and osmotic mechanism that determines the final concentration of lactose in milk.

Fixed effects

The greater LP in milk of primiparous than multiparous cows (Figure 1) agrees with findings of Haile-Mariam and Pryce (2017), and it could be attributed to a higher SCC level in milk of multiparous animals, which results in higher incidences of mastitis (Oltenacu and Broom, 2010) and therefore lower LP. Moreover, first-calving cows are usually less stressed than older cows, because they do not have a previous lactation and they theoretically can convert a higher amount of serum glycogen into lactose (Larsen and Moyes, 2015). Indeed, multiparous are more likely to suffer from negative energy balance than primiparous cows, as milk production increases with parity number. Lactation curves of LP were similar to lactation curves of MY and LY (Figure 2), and opposite to lactation curves of FP and PP, again supporting that LP is not affected by the amount of milk produced. The greater persistency of LP and LY in first- compared with later-parity cows are responsible for the greater persistency of MY in primiparous than multiparous animals (Haile-Mariam and Pryce, 2017). Least squares means of LP and LY were similar across calving seasons, suggesting that the significance of this effect can be mainly attributed to the high number of records available in the study rather than to an actual seasonal effect.

The second ANOVA with the inclusion of the fixed effect of classes of LP (Figure 3) confirmed that LP and FRP have an opposite trend (Hanuš *et al.*, 2010; Kedzierska-Matysek *et al.*, 2011). In addition, the highest value of SCS assessed in the low-lactose class corroborates the view that cows with on average lower milk LP are those with higher milk SCS, and thus they are more likely to be susceptible to mastitis. This finding is supported by previous investigation on the effect of high SCS and mastitis on milk composition (Lindmark-Månsson *et al.*, 2006; Vilas Boas *et al.*, 2017).

Heritability and repeatability

Heritability of LP (0.43 ± 0.03; Table 2) was similar to estimates reported by Miglior *et al.* (2007) for Canadian

Table 2 *Additive genetic (σ^2_a), across-lactation permanent environmental (σ^2_c), within-lactation permanent environmental (σ^2_w) and residual (σ^2_e) variances, heritability (h^2; SE in parentheses) and repeatability (t; SE in parentheses) of daily bovine milk composition and production traits, milk freezing point and somatic cell score (SCS) ($n = 59\,811$)*

Traits	σ^2_a	σ^2_c	σ^2_w	σ^2_e	h^2 (SE)	t (SE)
Composition (%)						
Lactose	0.0101	0.0026	0.0022	0.0088	0.43 (0.03)	0.63 (0.01)
Fat	0.1144	0.0337	0.0157	0.1615	0.35 (0.02)	0.50 (0.01)
Protein	0.0302	0.0062	0.0117	0.0267	0.40 (0.03)	0.64 (0.01)
Production (kg/day)						
Milk	3.3373	3.3522	7.5521	9.6665	0.14 (0.02)	0.60 (0.01)
Lactose	0.0080	0.0078	0.0176	0.0232	0.14 (0.02)	0.59 (0.01)
Fat	0.0033	0.0073	0.0115	0.0271	0.07 (0.02)	0.45 (0.01)
Protein	0.0024	0.0040	0.0063	0.0110	0.10 (0.02)	0.54 (0.01)
Freezing point (°C)	0.3833[1]	0.2659[1]	0.2807[1]	2.2731[1]	0.12 (0.01)	0.29 (0.01)
SCS (units)	0.2701	0.3479	0.7630	1.2232	0.10 (0.02)	0.53 (0.01)

[1]($\times 10^{-5}$).

Table 3 *Phenotypic correlations (above diagonal) and genetic correlations (below diagonal) between the studied traits in bovine milk*

Traits	MY	FY	PY	LY	FP	PP	LP	FRP	SCS
MY	–	0.70 (0.00)	0.89 (0.00)	0.98 (0.00)	−0.25 (0.01)	−0.35 (0.01)	0.06 (0.01)	0.09 (0.01)	−0.11 (0.01)
FY	0.06 (0.14)	–	0.75 (0.00)	0.69 (0.00)	0.48 (0.01)	0.01 (0.01)	0.04 (0.01)	−0.02 (0.01)	−0.06 (0.01)
PY	0.68 (0.06)	0.43 (0.11)	–	0.88 (0.00)	−0.05 (0.01)	0.08 (0.01)	0.07 (0.01)	−0.02 (0.01)	−0.07 (0.01)
LY	0.97 (0.00)	0.05 (0.14)	0.64 (0.06)	–	−0.25 (0.01)	−0.34 (0.01)	0.23 (0.01)	−0.01 (0.01)	−0.15 (0.01)
FP	−0.74 (0.06)	0.63 (0.08)	−0.21 (0.09)	−0.71 (0.06)	–	0.48 (0.01)	−0.02 (0.01)	−0.15 (0.01)	0.04 (0.01)
PP	−0.59 (0.06)	0.37 (0.10)	0.20 (0.09)	−0.57 (0.06)	0.74 (0.03)	–	−0.02 (0.01)	−0.25 (0.01)	0.09 (0.01)
LP	−0.02 (0.08)	0.00 (0.10)	0.01 (0.09)	0.28 (0.08)	0.01 (0.06)	−0.01 (0.06)	–	−0.53 (0.01)	−0.25 (0.01)
FRP	0.06 (0.10)	−0.14 (0.12)	−0.14 (0.11)	−0.07 (0.10)	−0.13 (0.07)	−0.24 (0.07)	−0.46 (0.05)	–	0.08 (0.01)
SCS	0.11 (0.12)	0.08 (0.14)	0.14 (0.13)	0.05 (0.12)	0.00 (0.09)	0.02 (0.09)	−0.22 (0.08)	0.14 (0.10)	–

MY = milk yield; FY = fat yield; PY = protein yield; LY = lactose yield; FP = fat percentage; PP = protein percentage; LP = lactose percentage; FRP = freezing point; SCS = somatic cell score.
SE are given in parentheses.

Holsteins and by Gillon *et al.* (2010) in a multi-breed study, and higher than heritabilities of 0.33 and 0.30 estimated in Holstein cows by Tiezzi *et al.* (2013) and Petrini *et al.* (2016), respectively, and 0.25 reported by Sneddon *et al.* (2015) in a multi-breed population. In agreement with findings of Tiezzi *et al.* (2013) and Haile-Mariam and Pryce (2017), a lower heritability of LY (0.14 ± 0.02) compared with LP was somewhat expected, as LY is strongly related to MY. Regarding FRP, Costa *et al.* (2018) estimated a heritability of 0.11 and a repeatability of 0.26 in a large data set of primiparous Holstein cows, very close to the values of the present research. Nevertheless, comparison with the literature is difficult because few genetic studies have attempted to estimate genetic parameters of FRP at population level. Overall, heritabilities of FP and PP were in agreement with estimates reported in Ireland by Visentin *et al.* (2017), and heritabilities of MY, FY and PY were in accordance with findings of Tiezzi *et al.* (2013) and Sneddon *et al.* (2015). The repeatabilities of LP (0.63 ± 0.01) and LY (0.59 ± 0.01) agreed with recent findings of Scarso *et al.* (2017) and Visentin *et al.* (2017), and suggested that ~40% of the variability of these traits is attributable to temporary environmental effects.

Phenotypic correlations

Similar to our findings, Hanuš *et al.* (2010) and Costa *et al.* (2017) assessed a negative phenotypic correlation between LP and FRP. The negative association between FRP and solids percentages (Table 3) corroborated that this trait is mainly associated with concentration of milk components, especially with water-soluble traits (lactose and protein; Fox *et al.*, 2015). Somatic cell score was negatively correlated with LP, as reported by Lindmark-Månsson *et al.* (2006) and Vilas Boas *et al.* (2017), and with LY, similar to the results of Haile-Mariam and Pryce (2017). Indeed, lactose synthesis and availability in udder decreases in presence of high SCC, due to mastitis pathogens which use lactose as a substrate, but also due to the increase of mammary homeostasis during inflammation (Blum *et al.*, 2008; Alessio *et al.*, 2016). Berglund *et al.* (2007) observed a decrease of LP from 4.86% to 4.69% when SCC increased from 31 000 to 450 000 cells/ml in Swedish Red and White cows.

Genetic correlations

Overall, genetic correlations (Table 3) were in agreement with estimates reported by Miglior *et al.* (2007), Sneddon *et al.* (2015) and Visentin *et al.* (2017). The weak genetic relationship

between LP and LY was expected as LP is the concentration of lactose in udder in equilibrium with water, whereas LY is the anabolic amount of lactose synthesized and present in a given volume of milk. Lactose yield is more dependent on blood glucose and on GLUT transporters expression; therefore, the correlation between LP and LY was expected to be positive but even far from unity. The correlation between LP and SCS was consistent with the estimates of Sneddon *et al.* (2015) and Haile-Mariam and Pryce (2017). Nevertheless, investigations including data of mastitis diagnoses are necessary to validate LP as reliable indicator of intramammary infection. The negative genetic correlation between LP and FRP was expected because milk FRP depends on milk solids concentration. Indeed, more positive FRP values (> −0.520°C) denote diluted milk. Considering this result and that low values of FRP are desirable, FRP could be depressed or stabilized through genetic selection for higher LP. In the present study, the correlation between LP and FP estimated through a repeatability animal model was close to zero. However, Haile-Mariam and Pryce (2017), using random regression models, estimated positive correlations between LP and FP at the beginning of lactation and negative at the end. Finally, the genetic correlation close to one between LY and MY corroborates that milk volume is highly dependent on lactose synthesis in the udder (Miglior *et al.*, 2007; Sneddon *et al.*, 2015; Haile-Mariam and Pryce, 2017). Therefore, selection indexes with a positive weight for MY such as in the United States and South Africa, indirectly, account for LY.

Conclusions

Lactose is a milk component of economic relevance and under substantial genetic control, exhibiting greater heritability than most traditional milk traits already included in selection indexes worldwide. Genetic selection for LP is thus feasible and genomic selection could be used on this trait to help improve milk marketability and maybe respond to new market demands. Correlations with traits of interest are present and therefore lactose could be used to accelerate the gains of those traits that are difficult and expensive to measure, and that exhibit low heritability. For example, after validation with mastitis information, LP could help in monitoring the udder health of dairy cows, in addition to SCC. Further work should be carried out to estimate the economic value of LP under different scenarios and to shift genetic selection towards country-specific needs.

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Declaration of interest

The authors declare that they do not have conflicts of interest.

Ethics statement

Procedures used in this study are excluded from the authorization of the animal welfare committee.

Software and data repository resources

None of the data were deposited in an official repository.

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Chapter II

Genetic relationships of lactose and freezing point with minerals and coagulation traits predicted from milk mid-infrared spectra in Holstein cows

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Genetic relationships of lactose and freezing point with minerals and coagulation traits predicted from milk mid-infrared spectra in Holstein cows

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ABSTRACT

The aim of the present study was to assess the relationships of lactose percentage (LP), lactose yield (LY), and freezing point (FRP) with minerals and coagulation properties predicted from mid-infrared spectra in bovine milk. To achieve this purpose, we analyzed 54,263 test-day records of 4,297 Holstein cows to compute (co) variance components with a linear repeatability animal model. Parity, stage of lactation, season of calving, and herd-test-date were included as fixed effects in the model, and additive genetic animal, within- and across-lactation permanent environment, and residual were included as random effects. Lactose percentage was more heritable (0.405 ± 0.027) than LY (0.121 ± 0.021) and FRP (0.132 ± 0.014). Heritabilities (± standard error) of predicted milk minerals varied from 0.375 ± 0.027 for Na to 0.531 ± 0.028 for P, and those of milk coagulation properties ranged from 0.348 ± 0.052 for rennet coagulation time to 0.430 ± 0.026 for curd firming time. Lactose percentage showed favorable (negative) genetic correlations with milk somatic cell score (SCS) and FRP, and it was almost uncorrelated with casein-related minerals (Ca and P) and coagulation properties. Moreover, LP was strongly correlated with Na (−0.783 ± 0.022), a mineral known to increase in the presence of intramammary infection (IMI) and high somatic cell count. Indeed, Na is the main osmotic replacer of lactose in mastitic milk when the blood–milk barrier is altered during IMI. Being strongly associated with milk yield, LY did not favorably correlate with coagulation properties, likely because of the negative correlation of this trait with protein and casein percentages. Milk FRP presented moderate and null genetic associations with Na and SCS, respectively. Results of the present study suggest that the moderate heritability of LP and its genetic correlations with IMI-related traits (Na and SCS) could be exploited for genetic selection against mastitis. Moreover, selection for LP would not impair milk coagulation characteristics or Ca and P content, which are important for cheesemaking.
Key words: lactose, milk mineral, cheesemaking, genetic correlation, somatic cell count

INTRODUCTION

Milk lactose has gained interest in the scientific community in recent years and some studies have investigated genetic and nongenetic sources of variation for this trait (Sneddon et al., 2015; Haile-Mariam and Pryce, 2017; Costa et al., 2019b). Lactose is the major milk osmotic compound and thus the main compound responsible for milk volume (Fox et al., 2015); this means that it would not be biologically possible to strongly reduce lactose percentage (**LP**) through, for example, genetic selection and feeding strategies, to meet requirements of people intolerant to lactose. In contrast to fat and protein concentrations, LP is not affected by milk dilution through DIM, and its lactation curve mirrors that of milk yield (**MY**) and lactose yield (**LY**), with a peak in early lactation (Haile-Mariam and Pryce, 2017). When IMI occurs, the inflammatory response factors of white cells are responsible for damaging the epithelium surrounding alveoli. This alters the mammary blood barrier equilibrium, causing a decrease of LP in the alveoli, which is osmotically balanced by some blood minerals (mainly Na and Cl). This mechanism explains why mastitic milk has greater electrical conductivity, lower LP, and a saltier taste than milk of healthy cows (Norberg et al., 2006; Brandt et al., 2010; Fox et al., 2015). Supporting this, an inverse relationship exists between LP and SCS, the latter being the most common indicator of IMI and mastitis in dairy cows (Damm et al., 2017; Shook et al., 2017). For example, Miglior et al. (2007) estimated a genetic correlation of −0.20 between LP and SCS in

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Canadian Holsteins and Gillon et al. (2010) reported a higher negative value (−0.38) in a multi-breed study in Belgium. By adopting a random regression model, Haile Mariam and Pryce (2017) observed an increase in the magnitude of the genetic correlation between these 2 traits in late lactation of multiparous cows. Indeed, they estimated a genetic correlation close to −0.50 in third-lactation cows at 300 DIM. The strongest phenotypic (−0.66) and genetic (−0.44) associations between LP and SCS were assessed by Vilas Boas et al. (2017) in Brazilian Gyr cows and by Stoop et al. (2007) in first-calving Holsteins, respectively. Miglior et al. (2007) and Haile-Mariam and Pryce (2017) estimated negative correlations (−0.16 and −0.15, respectively) between LP EBV and SCS EBV, highlighting that bulls with low EBV for LP are more likely to produce offspring with higher SCS; that is, more susceptible to mastitis. In addition, Costa et al. (2019a) estimated a negative genetic correlation (−0.175) between LP and mastitis in Austrian Fleckvieh cattle. These findings, coupled with moderate to high heritability of LP (e.g., Stoop et al., 2007; Tiezzi et al., 2013; Costa et al., 2019a,b), make this trait an interesting indicator for the genetic improvement of cow udder health.

The variation of milk composition following an IMI, especially in terms of LP and mineral content, is thought to affect milk freezing point (**FRP**, °C). Milk FRP is closely dependent on water-soluble compounds and it is often used as an alert to the fraudulent addition of water to milk; indeed, milk with FRP values greater than −0.520°C (i.e., values moving toward zero) are considered unsuitable for human consumption (European Union, 1992; Council Directive 92/46/EEC). From a genetic point of view, information on genetic parameters for FRP at the population level is scarce (Costa et al., 2019b).

The potential use of LP and FRP for selection purposes in the Italian production system depends on the magnitude and direction of genetic relationships with traits of economic relevance for the dairy industry, such as milk minerals and milk coagulation properties (**MCP**). Indeed, 82.2% of Italian bovine milk (11,950,245 t in 2017) is destined for cheese manufacture (CLAL, 2017, 2018; ISTAT, 2017); thus, milk minerals (especially Ca and P) and MCP play a key role in the efficiency of milk transformation. In Italy, MCP have economic relevance and are included in the payment systems of Parmigiano Reggiano (Summer et al., 2015) and Trentingrana (Penasa et al., 2016b) cheeses to reward or penalize farmers for milk coagulation ability. Routine determination of mineral contents and MCP through reference laboratory methods in individual milk samples is expensive, and the use of mid-infrared spectroscopy to predict these traits from available mid-infrared spectra is a cheaper and faster approach to collect information at the population level (Soyeurt et al., 2009; Visentin et al., 2016). Therefore, the aim of the present study was to quantify phenotypic and genetic correlations of milk LP, LY, and FRP with SCS, predicted minerals, and predicted MCP in Holstein cows.

MATERIALS AND METHODS

Data and Editing

A data set of 143,965 test-day records of 11,230 Holstein cows in 676 herds of Bolzano province (northern Italy) was available for statistical investigation. Milk samples were collected from January 2011 to December 2014 during the routine recording scheme and analyzed in the laboratory of the South Tyrolean Dairy Association (Südtiroler Sennereiverband, Bolzano, Italy) for LP, fat percentage (**FP**), protein percentage (**PP**), CN percentage, and FRP using a Milkoscan FT6000 (Foss Electric A/S, Hillerød, Denmark). Somatic cell count (cells/μL) was determined using Fossomatic FC (Foss Electric A/S) and converted to SCS to achieve normal distribution by applying the formula of Wiggans and Shook (1987): $SCS = \log_2(SCC/100) + 3$. Rennet coagulation time (**RCT**, min), curd firmness 30 min after rennet addition ($\mathbf{a_{30}}$, mm), curd firming time ($\mathbf{k_{20}}$, min), and Ca, P, Mg, Na, and K were predicted from the same data set using stored spectra information and the mid-infrared prediction models developed and validated by Visentin et al. (2016). Coefficients of determination in external validation of prediction models for Ca, P, Mg, K, and Na were 0.67, 0.68, 0.65, 0.69, and 0.40, respectively, and for RCT, k_{20}, and a_{30} they were 0.54, 0.56, and 0.52, respectively (Visentin et al., 2016).

Cows were retained in the data set if they had known sire and dam, were in parity 1 to 6, and were between 6 and 480 DIM. The range of age at calving within each parity was 20 to 40, 32 to 58, 44 to 76, 56 to 94, 68 to 112, and 75 to 120 mo for first-, second-, third-, fourth-, fifth-, and sixth-parity animals, respectively. Moreover, values of MY, composition, FRP, minerals, and MCP deviating more than 3 standard deviations from the respective means were treated as missing, whereas records characterized by SCC <1,000 or >15,000,000 cells were discarded. Lactose yield was calculated by multiplying LP and MY. Finally, at least 3 records per cow within lactation and at least 3 observations per contemporary group (herd-test-date, **HTD**) were guaranteed. The average number of records per cow within lactation in the final data set was 7 and ranged from 3 to 17.

Genetic Parameters

To limit computational memory and time, genetic parameters of the studied traits were estimated on a randomly selected subset of 40% of herds (n = 269), which resulted in 54,263 records from 4,297 cows, daughters of 784 sires and 3,484 dams. Means and variation of the traits were very similar in the 2 sets. Also, the frequency of observations across parities of the subset (31.24, 27.91, 18.78, 12.23, 6.49, and 3.35% for first, second, third, fourth, fifth, and sixth parity, respectively) mirrored those of the whole data set (31.19, 27.90, 18.88, 11.88, 6.67, and 3.48%). Six generations of ancestors were traced back, leading to a pedigree file of 16,925 individuals. Variance and covariance components were estimated in ASReml 4.1 (Gilmour et al., 2015) using single-trait and bivariate repeatability animal models, respectively. The general form of the model, in matrix notation, was as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_{\mathrm{p}}\mathbf{p} + \mathbf{Z}_{\mathrm{w}}\mathbf{w} + \mathbf{Z}_{\mathrm{a}}\mathbf{a} + \mathbf{e},$$

where $\mathbf{y}$ is the vector of phenotypic records for MY, LY, LP, CN, PP, FP, FRP, SCS, Ca, P, Mg, K, Na, RCT, a_{30}, and k_{20}; $\mathbf{b}$ is the vector of fixed effects of contemporary group (HTD: 6,622 levels), parity order (1 to 6), season of calving (4 classes: December to February, March to May, June to August, and September to November), and stage of lactation [13 classes of 30 d each, except for the first class (6 to 30 DIM), the second to last class (330 to 390 DIM), and the last class (391 to 480 DIM)]; $\mathbf{p}$ is the vector of solutions for random permanent environmental effect across lactation; $\mathbf{w}$ is the vector of solutions for random permanent environmental effect within lactation; $\mathbf{a}$ is the vector of solutions for random additive genetic effect of the animal; $\mathbf{e}$ is the vector of random residuals; and $\mathbf{X}$, $\mathbf{Z}_{\mathrm{p}}$, $\mathbf{Z}_{\mathrm{w}}$, and $\mathbf{Z}_{\mathrm{a}}$ are incidence matrices relating the corresponding effects to the dependent variable. Random effects were assumed to be normally distributed with null means and variance-covariance structures of additive genetic, permanent environmental across-lactation, permanent environmental within-lactation, and residual effects in the bivariate analyses that were equal to $\mathbf{G} \otimes \mathbf{A}$, $\mathbf{P} \otimes \mathbf{I}$, $\mathbf{W} \otimes \mathbf{I}$, and $\mathbf{R} \otimes \mathbf{I}$, respectively, where $\mathbf{G}$ is the 2×2 additive genetic (co)variance matrix, $\mathbf{P}$ is the 2×2 (co)variance matrix of permanent environmental effects across lactation, $\mathbf{W}$ is the 2×2 (co)variance matrix of permanent environmental effects within lactation, $\mathbf{R}$ is the residual (co)variance matrix, $\mathbf{A}$ is the additive genetic relationship matrix among individuals, $\mathbf{I}$ is an identity matrix of appropriate order, and $\otimes$ denotes the Kronecker product. The phenotypic variance was the sum of additive genetic, permanent environmental across-lactation, permanent environmental within-lactation, and residual variances. Heritability was computed as the ratio of the additive genetic variance to the phenotypic variance, and repeatability was the ratio of the sum of additive genetic and permanent environmental variances to the phenotypic variance. Genetic (or phenotypic) correlations (r) were calculated as

$$\mathrm{r} = \frac{cov_{1,2}}{\sqrt{\sigma_1^2 \times \sigma_2^2}},$$

where $cov_{1,2}$ is the additive genetic (or phenotypic) covariance between traits 1 and 2, and σ_1^2 and σ_2^2 are the additive genetic (or phenotypic) variances of trait 1 and trait 2, respectively.

RESULTS AND DISCUSSION

Descriptive Statistics

Lactose percentage ranged from 4.06 to 5.46% and averaged 4.76% across all 6 parities (Table 1). The average LP was equal to the value reported by Scarso et al. (2017) in a multi-breed study in Ireland and slightly lower than the mean LP reported by Sneddon et al. (2015) in a data set including Holstein, Jersey, and other minor breeds reared in New Zealand, and by Haile-Mariam and Pryce (2017) for Australian Holstein and Jersey cows. The mean LP of the present study was lower than the average value (4.90%) reported by Penasa et al. (2016a) for the same breed in Italy; however, this difference was expected because the study of Penasa et al. (2016a) included only primiparous cows, whose milk has greater LP than milk of multiparous cows (Haile-Mariam and Pryce, 2017). This was also the case in the present study; indeed, primiparous cows produced milk with greater LP (4.82%) than did later parity cows (4.76% for second-parity to 4.71% for sixth-parity cows). Lactose yield averaged 1.31 kg/d and varied from 0.17 to 2.61 kg/d (Table 1). Tiezzi et al. (2013) reported a greater mean value (1.55 kg/d) using data of Italian Holsteins, and Sneddon et al. (2015) calculated a lower value (0.71 kg/d) in New Zealand. Milk FRP averaged −0.525°C with a coefficient of variation (**CV**) of 1.90% (Table 1), and it averaged −0.527, −0.525, and −0.524°C in first, second, and third parity, respectively, and −0.523 in later parities. Hanuš et al. (2010) reported lower mean (−0.532°C) and CV (0.90%) for FRP in a multi-breed study. Overall, means of predicted milk minerals (Table 1) were in agreement with those reported by Buitenhuis

Table 1. Summary statistics of milk traits

Trait	n	Mean	Minimum	Maximum	CV, %
Yield, kg/d					
Milk	143,343	27.45	3.70	51.00	27.87
Lactose	143,343	1.31	0.17	2.61	28.57
Composition, %					
Lactose	143,965	4.76	4.06	5.46	3.36
Casein	143,588	2.66	1.84	3.53	10.53
Protein	143,569	3.38	2.22	4.53	11.24
Fat	143,567	4.02	2.10	5.94	15.67
Freezing point, °C	143,965	−0.525	−0.552	−0.498	1.90
SCS	143,965	2.92	−3.64	10.22	60.27
Mineral content, mg/kg					
Calcium	143,098	1,317.00	823.10	1,821.15	12.03
Phosphorus	143,435	928.76	600.68	1,258.75	11.48
Magnesium	125,078	138.47	62.29	193.87	19.14
Potassium	143,003	1,505.24	1,102.00	1,909.11	8.54
Sodium	143,642	427.05	273.52	581.59	11.75
Milk coagulation properties					
Rennet coagulation time, min	135,130	23.25	4.67	32.25	16.00
Curd firming time, min	143,854	6.07	0.30	10.00	20.43
Curd firmness, mm	121,092	16.14	0.003	65.25	57.31

et al. (2015) for milk minerals measured using the reference method in Danish Holsteins and by Visentin et al. (2018) for infrared-predicted milk minerals in dairy and dual-purpose cattle breeds. The CV of milk minerals were comparable to those of FP, PP, and CN, and they ranged from 8.54% (K) to 19.14% (Mg). Means for RCT, k_{20}, and a_{30} were 23.25 min, 6.14 min, and 16.07 mm, respectively, with a_{30} exhibiting much greater CV (57.31%) than RCT (16.00%) and k_{20} (20.43%), in agreement with Cassandro et al. (2015).

Additive Genetic Variation, Heritability, and Repeatability

Genetic parameters for milk minerals and MCP in the present study were estimated using predictions from models that were moderately accurate, and estimates have to be interpreted with caution, especially with regard to Na, which was the least predictable trait. Nevertheless, mid-infrared predictions could be used as indicator traits at the population level to drive breeding strategies. For example, Cecchinato et al. (2009) reported coefficients of determination from 0.61 to 0.69 for RCT models, and from 0.46 to 0.52 for a_{30} models, which were similar to accuracies of prediction models for RCT and a_{30} of Visentin et al. (2016). Despite the moderate phenotypic correlations between measured and predicted MCP, their genetic counterparts were stronger (Cecchinato et al., 2009), supporting the role of mid-infrared predictions as indicator traits in breeding programs aiming to improve milk coagulation ability. It is likely that genetic correlations between measured and predicted milk minerals are also greater than their phenotypic counterparts, but unfortunately we could not estimate such genetic relationships in the current study because the reference data set was small for this purpose.

The coefficient of additive genetic variation ($\mathbf{CV_a}$) of LP was lower (1.95%) than that of other milk traits, except FRP (0.40%; Table 2), and was very close to that (1.89%) reported by Visentin et al. (2017) for the same trait in a multi-breed study. This finding was expected because lactose has a physiological osmotic function at the mammary cell level and its content in milk does not exhibit wide variation. The CV_a of LY (6.35%) was close to that of MY (6.18%), and for minerals it ranged from 3.98% for K to 6.71% for P. The greatest CV_a was obtained for a_{30} (28.65%) and SCS (19.80%; Table 2).

Lactose percentage was more heritable (0.405) than LY (0.121). Similarly, Scarso et al. (2017) and Visentin et al. (2017) estimated heritabilities for LP that were in agreement with those obtained in the present study. The heritability of LP was similar to that of FP, PP, and CN but much greater than that assessed for MY and SCS. Milk FRP was lowly heritable (0.132) and repeatable (0.267), indicating that its variation depends mainly on temporary environmental rather than on genetic and permanent environmental factors (Costa et al., 2019b). Among milk minerals, the least and most heritable were Na (0.375) and P (0.531), respectively, with Ca, Mg, and K exhibiting intermediate heritability of approximately 0.45 (Table 2). These estimates were slightly lower than those assessed by van Hulzen et al. (2009) for Ca (0.57), P (0.62), and Mg (0.60) using the reference method in 1,948 milk samples of Dutch Friesian cows, and in agreement with estimates of Soy-

eurt et al. (2012) for infrared-predicted Ca (0.50), P (0.55), and Mg (0.52) in milk of first-calving Holsteins. Repeatabilities of milk minerals were moderately high, between 0.558 (Ca) and 0.686 (P), highlighting that they were less affected by temporary environmental effects than by other traits. Estimates of heritability and repeatability for MCP ranged from 0.348 (RCT) to 0.430 (k_{20}) and from 0.564 (a_{30}) to 0.591 (k_{20}), respectively (Table 2). Standard errors of heritability and repeatability estimates ranged from 0.014 to 0.052 and 0.006 to 0.012, respectively (Table 2).

The proportion of phenotypic variance explained by permanent environmental effects was heterogeneous among traits, and it ranged from 11.2% (Ca) to 46.6% (MY). In particular, this proportion was much larger for LY (45.5%) than for LP (20.2%), and it was small for milk minerals, with values between 11.2% (Ca) and 23.3% (Na); the latter results could be due to the nature of milk minerals, which are physiologically determined and less affected by permanent environmental effects. Similar to milk minerals, FRP was marginally influenced by temporary and permanent environmental effects.

Correlations

Standard errors of genetic ($\mathbf{r_a}$) and phenotypic ($\mathbf{r_p}$) correlations estimated in the present study ranged from 0.009 to 0.117 and from 0.000 to 0.013, respectively (Table 3). The negative and strong r_a between LP and Na (−0.783; Table 3) emphasized the link between LP and this mastitis-associated mineral (Fox et al., 2015). Indeed, Na has been found to be positively related to milk SCC (Ogola et al., 2007; Soyeurt et al., 2008; Haron et al., 2014). The r_a between LP and K was negative (−0.432), similar to results of Visentin et al. (2018), who estimated r_a from −0.62 to −0.46 between LP and K using random regression models. The r_a between LP and Mg content was moderately weak (−0.275) and was almost null between LP and Ca, and between LP and P content. Except for the weak correlation with k_{20} (−0.118), LP was not genetically associated with MCP, suggesting that potential selection for LP would not impair milk coagulation ability. Using multivariate factor analysis, Macciotta et al. (2012) identified a factor named "udder health" (high LP and low SCC) and estimated its heritability (0.137) and r_a with MY (0.132), composition (0.126), acidity (0.168), and coagulation ability (0.008). The r_a between LP and SCS was −0.172 (Table 3). Despite the weak correlation, this finding suggested that genetic selection for increased LP would result, on average, in a reduction of SCS. Gillon et al. (2010) reported r_a of −0.35 between LP and SCS in a multi-breed study, and Miglior et al. (2007) and Visentin et al. (2017) estimated values of −0.20 and −0.28 in Canadian Holsteins and Irish mixed-breed herds, respectively. A stronger r_a (−0.44) was reported by Stoop et al. (2007) for Holstein cows in the Netherlands. Overall, the magnitude and direction of r_p resembled those of r_a (Table 3). In particular, LP was

Table 2. Coefficient of genetic variation (CV_a), heritability (h^2), repeatability (t), and proportion of phenotypic variance explained by the 2 permanent environmental effects (c^2) for milk yield and composition traits, freezing point, SCS, mineral contents, and coagulation properties

Trait	n	CV_a, %	h^2 (SE)	t (SE)	c^2
Yield, kg/d					
Milk	54,041	6.18	0.119 (0.021)	0.585 (0.006)	0.466
Lactose	54,041	6.35	0.121 (0.021)	0.576 (0.006)	0.455
Composition, %					
Lactose	54,263	1.95	0.405 (0.027)	0.607 (0.007)	0.202
Casein	54,086	5.16	0.430 (0.027)	0.642 (0.007)	0.212
Protein	54,089	5.30	0.423 (0.027)	0.625 (0.007)	0.202
Fat	54,113	8.02	0.345 (0.025)	0.511 (0.008)	0.166
Freezing point, °C	54,263	0.40	0.132 (0.014)	0.267 (0.007)	0.135
SCS	54,263	19.80	0.131 (0.019)	0.516 (0.006)	0.385
Mineral content, mg/kg					
Calcium	53,919	5.59	0.446 (0.024)	0.558 (0.008)	0.112
Phosphorus	54,075	6.71	0.531 (0.028)	0.686 (0.007)	0.155
Magnesium	47,171	5.13	0.470 (0.027)	0.602 (0.008)	0.132
Potassium	53,877	3.98	0.450 (0.025)	0.582 (0.008)	0.132
Sodium	54,150	5.92	0.375 (0.027)	0.608 (0.007)	0.233
Milk coagulation properties					
Rennet coagulation time, min	50,984	7.57	0.348 (0.052)	0.589 (0.012)	0.241
Curd firming time, min	54,218	10.25	0.430 (0.026)	0.591 (0.007)	0.161
Curd firmness, mm	46,032	28.65	0.385 (0.026)	0.564 (0.008)	0.179

Table 3. Genetic (r_a) and phenotypic (r_p) correlations of lactose percentage and yield and freezing point with all investigated traits

Trait	Lactose, %		Lactose, kg/d		Freezing point, °C	
	r_a (SE)	r_p (SE)	r_a (SE)	r_p (SE)	r_a (SE)	r_p (SE)
Yield, kg/d						
Milk	−0.113 (0.086)	0.058 (0.011)	0.954 (0.009)	0.986 (0.000)	0.176 (0.100)	0.090 (0.008)
Lactose	0.189 (0.083)	0.220 (0.010)	—	—	0.051 (0.102)	0.006 (0.008)
Composition, %						
Lactose	—	—	0.189 (0.083)	0.220 (0.010)	−0.411 (0.055)	−0.509 (0.007)
Casein	0.047 (0.055)	0.043 (0.013)	−0.661 (0.059)	−0.338 (0.009)	−0.321 (0.061)	−0.287 (0.008)
Protein	−0.031 (0.055)	−0.042 (0.013)	−0.674 (0.058)	−0.368 (0.009)	−0.295 (0.062)	−0.246 (0.008)
Fat	−0.008 (0.058)	−0.040 (0.012)	−0.771 (0.056)	−0.266 (0.009)	−0.219 (0.068)	−0.156 (0.008)
Freezing point, °C	−0.411 (0.055)	−0.509 (0.007)	0.051 (0.102)	0.006 (0.008)	—	—
SCS	−0.172 (0.076)	−0.255 (0.010)	−0.064 (0.117)	−0.166 (0.009)	0.008 (0.094)	0.068 (0.008)
Mineral content, mg/kg						
Calcium	−0.020 (0.051)	0.049 (0.012)	−0.401 (0.071)	−0.248 (0.010)	−0.178 (0.061)	−0.202 (0.008)
Phosphorus	−0.042 (0.052)	0.041 (0.013)	−0.464 (0.071)	−0.228 (0.011)	−0.260 (0.061)	−0.225 (0.009)
Magnesium	−0.275 (0.049)	−0.252 (0.012)	−0.624 (0.063)	−0.276 (0.010)	−0.105 (0.065)	−0.001 (0.009)
Potassium	−0.432 (0.043)	−0.340 (0.011)	0.178 (0.081)	−0.004 (0.011)	0.097 (0.064)	0.134 (0.009)
Sodium	−0.783 (0.022)	−0.784 (0.005)	0.234 (0.086)	−0.117 (0.011)	0.459 (0.055)	0.472 (0.007)
Milk coagulation properties						
Rennet coagulation time, min	−0.020 (0.054)	−0.125 (0.012)	0.120 (0.085)	−0.035 (0.011)	−0.048 (0.067)	0.042 (0.009)
Curd firming time, min	−0.118 (0.052)	−0.149 (0.012)	0.509 (0.071)	0.205 (0.010)	0.376 (0.058)	0.301 (0.008)
Curd firmness, mm	0.032 (0.055)	0.120 (0.012)	−0.258 (0.083)	−0.063 (0.011)	−0.058 (0.068)	−0.138 (0.009)

negatively correlated with Mg (−0.252), K (−0.340), and Na (−0.784). Lactose percentage was negatively phenotypically associated with SCS (−0.255) and FRP (−0.509), and it was weakly but favorably phenotypically related to RCT (−0.125), k_{20} (−0.149), and a_{30} (0.120). Bezman et al. (2015) and Bland et al. (2015) reported favorable and moderate r_p of LP with RCT and a_{30}. These findings pointed out the positive role of milk lactose in the fermentation activities of lactic acid bacteria before and during cheesemaking.

Lactose percentage was weakly associated with MY and composition, in accordance with Sneddon et al. (2015), who estimated r_a between −0.14 (LP with PP) and −0.05 (LP with FP), and Visentin et al. (2017), who reported r_a between −0.22 (LP with PP) and 0.07 (LP with MY). By adopting random regression models, Haile-Mariam and Pryce (2017) estimated r_a of LP with PP and FP that were positive at the beginning of the lactation, with estimates between 0.30 and 0.35 (LP with PP) and 0.15 to 0.20 (LP with FP), and negative at the end of the lactation with estimates from −0.25 to −0.10 (LP with PP) and −0.20 to −0.05 (LP with FP). These findings may explain the overall average weak correlations between LP and milk composition when estimated using a repeatability animal model.

The r_a between LP and LY was weakly positive (0.189; Table 3), which was somewhat expected because LP is osmotically determined during water uptake and LY is the product of LP and MY. The amount of synthesized lactose determines the volume of milk in mammary tissue; thus, a strong r_a (0.954) was assessed between LY and MY, in accordance with the literature (Sneddon et al., 2015; Haile-Mariam and Pryce, 2017). Moreover, the strong negative r_a of LY with FP (−0.771), PP (−0.674), and CN (−0.661) underlined that cows producing a high amount of lactose (i.e., high MY) were those producing a more diluted milk. Lactose yield was unfavorably genetically associated with RCT (0.120), k_{20} (0.509), and a_{30} (−0.258), and this could be due to the aforementioned strong negative genetic relationship between LY and P or CN. Also, unfavorable genetic correlations were assessed between LY and casein-related minerals, with estimates from −0.624 (LY and Mg) to −0.401 (LY and Ca; Table 3), confirming that genetic selection for higher LY (i.e., higher MY) could be detrimental for milk technological traits. Indeed, milk from high-producing cows is usually characterized by lower concentrations of constituents and poorer coagulation properties (Cassandro et al., 2008). Positive weak r_a were assessed between LY and Na (0.234) and between LY and K (0.178). This finding indicated that cows yielding more lactose (and thus more milk) genetically tend to present higher milk Na content, which could be due to the greater susceptibility to mastitis and IMI

that characterizes high-producing cows (Oltenacu and Broom, 2010). However, LY correlated weakly with SCS (−0.064). Overall, r_p between LY and other traits investigated in the present study were lower than and of the same direction as their genetic counterparts, with very few exceptions (Table 3). In particular, r_p between LY and minerals ranged from −0.276 (LY and Mg) to −0.004 (LY and Na), and they were even lower between LY and MCP.

Milk FRP depends mainly on the concentration of the different constituents in milk (Fox et al., 2015). Indeed, in the current study, FRP was favorably genetically associated with composition traits (−0.411 with LP to −0.219 with FP) and contents of Ca (−0.178), P (−0.260), and Mg (−0.105; Table 3). These estimates were very similar to those obtained for the r_p and in agreement with previous reports (Harris and Bachman, 2003; Fox et al., 2015). Freezing point was moderately genetically related to Na (0.459) and k_{20} (0.376) and almost uncorrelated with K content, RCT, and a_{30}. Both r_a and r_p between FRP and Na suggested that milk with a higher content of Na (a potential indicator of IMI) usually presents undesired (i.e., greater) values of FRP. Following this reasoning, we would expect a positive correlation between FRP and SCS; however, this was not the case, because the 2 traits did not correlate in the present study. Somatic cell score and clinical mastitis are not always strongly correlated (r_a from 0.62 to 0.74; Egger-Danner et al., 2015) and test-day SCS is an alert for subclinical and chronic cases, rather than of (sub-)acute inflammations because peaks could be missed during the gap (3 to 4 wk) between consecutive test days (Koeck et al., 2010). Moreover, according to the pathogen involved, the increase in SCS differs in terms of pattern, persistency, and rapidity (Heringstad et al., 2000; de Haas, 2003). Finally, it is worthwhile noting that the osmotic equilibrium of milk tends to be guaranteed in the presence of mastitis or high SCS, the decrease in LP being replaced by mineral constituents (Fox et al., 2015). Therefore, further research is needed to investigate genetic (co)variance components of milk FRP across DIM.

To assess the consistency of genetic and phenotypic correlations of the most innovative traits (i.e., milk mineral contents and MCP) across different subsets, 2 additional samples, each comprising 40% of herds, were randomly selected from the original data and compared with estimates of the subset discussed in the present study (Supplemental Table S1; https://doi.org/10.3168/jds.2018-15378). Results highlighted trivial differences between the same phenotypic correlations estimated in the 3 subsets. In terms of genetic relationships, the differences were greater, especially for associations that involved LY; nevertheless, the magnitude of standard errors suggested that estimates were not significantly different across the 3 subsets.

CONCLUSIONS

Genetically, LP of bovine milk was favorably associated with FRP and almost uncorrelated with casein-related minerals and coagulation properties. Both phenotypic and genetic correlations of LP with common milk indicators of mastitis (SCS and, in particular, Na) exist and thus LP could be exploited as an additional indicator of udder health for indirect selection against mastitis. Supported by results of the present study, selecting for higher LP would not impair milk coagulation ability at the population level, whereas selection for FRP would negatively affect k_{20}. Milk minerals with nutritional and technological value (Ca, P, and Mg) would not be affected by selection for higher LP and lower FRP. Finally, we found no evidence to indicate that an indirect undesired response in traditional milk quality traits (e.g., fat, protein, and casein percentages) would result in case of selection for LP.

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Chapter III

Genetic associations of lactose and its ratios to other milk solids with health traits in Austrian Fleckvieh cows

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ABSTRACT

The genetic correlations (r_a) of milk lactose percentage (LP), lactose yield (LY), and ratios of LP to other milk solids with udder, metabolic, and fertility disorders have not been assessed in dairy cattle so far. To evaluate the potential role of milk lactose as indicator of cow health, 142,285 lactation records of 84,289 Austrian Fleckvieh cows were analyzed with univariate and bivariate animal models. Milk traits were on a 150-d basis and health traits were coded as binary (0/1). Other than LP and LY, 3 new phenotypes were defined and included in the present study, namely the lactose-to-fat, lactose-to-protein, and lactose-to-solids ratios. The most heritable trait was LP (0.566 ± 0.008) and heritability of LY was much lower (0.145 ± 0.005). Heritability estimates close to 0.50 were assessed for the ratios. The frequency of health disorders was higher in multiparous cows yielding milk with low LP (≤4.553%) compared with cows yielding milk with high LP (≥5.045%). Heritabilities of health traits were in the expected ranges, with the highest estimate for ovarian cysts (CYS; 0.037 ± 0.004) and the lowest for retained placenta (0.005 ± 0.001). Mastitis (MAS) genetically correlated with LY (0.518 ± 0.057); considering that the amount of synthesized lactose is the key regulator of milk volume, this result confirmed that high-producing cows are more genetically susceptible to MAS than low-producing animals. Similar to MAS, ketosis (KET) was also positively genetically associated with LY (0.420 ± 0.077) and a weak and unfavorable r_a between KET and lactose-to-protein ratio was estimated (0.159 ± 0.077). The r_a of LY with milk fever (MFV) and CYS were approximately 0.20. The r_a of LP with MAS, KET, and MFV were negative (−0.142 on average), supporting the idea that LP is a potential health indicator. Genetic correlations between health traits ranged from zero (retained placenta with MAS and CYS) to 0.463 ± 0.090 (MAS and MFV). Results of the present study suggest that LP has potentiality to be used as indicator trait to improve udder health in Austrian Fleckvieh population.

Key words: cattle, lactose, mastitis, fertility, genetic correlation

INTRODUCTION

Dairy cow health has become a target of several breeding programs worldwide, with increased weight in selection indexes (Pfeiffer et al., 2015; Fuerst-Waltl et al., 2016; Pryce et al., 2016). Apart from consumer sensitivity to animal welfare, concerns of farmers and the scientific community about impaired cows' productivity and performance are reasons for this increased interest (Egger-Danner et al., 2010; Oltenacu and Broom, 2010). In fact, health and metabolic status of cows affect milk yield (**MY**), composition, and technological properties (Leitner et al., 2011; Cinar et al., 2015; Fox et al., 2015). Economic losses associated with major diseases affecting dairy cows are usually indirect and differ among countries (Gonçalves et al., 2018; Hadrich et al., 2018; Mostert et al., 2018). A case of mastitis (**MAS**) costs around €300 in Europe, with substantial differences according to the severity (ICAR, 2018). In Austria, the cost of a MAS case is slightly higher (€341), and it includes milk embargo due to medical treatments (Fuerst-Waltl et al., 2016); however, considering all outflows, peaks of €600 per single MAS case have been reported in Austrian dairy farms (Braunvieh Austria, 2018). Rollin et al. (2015) estimated a cost of $444 per case of MAS occurring in the first 30 DIM in US dairy cattle. Moreover, Gohary et al. (2016) estimated an economic loss of US$203 per case of subclinical ketosis in Canada, and Mostert et al. (2018) reported that health costs generally increase with parity order.

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Considering that the genetic variance and heritability of most common diseases are low, the use of correlated indicator traits in milk could be beneficial to speed up genetic responses by increasing the accuracy of breeding values (Koeck et al., 2012; Egger-Danner et al., 2015; Pryce et al., 2016). Lactose, the main solid in bovine milk, has been recently proposed as a potential indicator trait of both udder health and metabolic status of the cow (Haile-Mariam and Pryce, 2017; Løvendahl and Riis Weisbjerg, 2017; Ebrahimie et al., 2018). Lactose derives from blood sugars, and it has been estimated that the udder uses 60 to 85% of circulating glucose in ruminants for lactose synthesis (Zhao, 2014). In high-producing dairy cattle, the amount of synthesized lactose can exceed 4.4 kg/d (Aschenbach et al., 2010). The lactation curve of lactose yield (**LY**) resembles that of MY and, thus, blood glucose uptake by udder is maximum at the peak of lactation. The LY produced by the mammary gland is in charge of determining water uptake from the cytosol and thus of milk volume.

Negative energy balance, and consequently ketosis (**KET**), arises when cows are not able to cope with the high demands of milk production, mainly because the absorption of glucose from the intestine by sodium-dependent glucose transporters is often not enough to cover the metabolic demand of dairy cows, especially at the beginning of lactation (Aschenbach et al., 2010). Several authors reported that KET decreases both LY and lactose percentage (**LP**; Cant et al., 2002; Reist et al., 2002; Fox et al., 2015; Larsen and Moyes, 2015); however, in high-producing cows, MY is the main anabolic scope, and the udder demand for glucose is a priority (Aschenbach et al., 2010), as the deficit of blood glucose may be counterbalanced by other sources; namely, greater conversion of AA into glucose in liver and reduced delivery of glucose in nonmammary tissues. Therefore, glucose availability at the udder level is maintained and LY and MY are not strongly impaired even in the presence of negative energy balance (Aschenbach et al., 2010). Although genetic and phenotypic mechanisms relating to blood sugar and milk sugar are not fully known, high availability of blood glucose, positive metabolic energy status of cow, and good expression of glucose transporters are always translated into high LY and LP (Ouweltjes et al., 2007). Indeed, LP was found to be 15% lower in cows fed a low-energy diet (Beerda et al., 2007) and negatively related to milk BHB (Larsen and Moyes, 2015), a ketone body derived from liver gluconeogenesis from fatty acids and used as indicator of KET (Larsen and Moyes, 2015). Lactose percentage is the physiological point at which the osmotic equilibrium between milk and blood is reached and, therefore, the call of water by udder from circulatory system stops. Nevertheless, to our knowledge only Belay et al. (2017) investigated the relationships of KET with milk LP and LY in Norwegian Red cows. In their study, phenotypic and genetic correlations with LP and phenotypic correlation with LY were close to zero, whereas the genetic correlation with LY was 0.16. This finding supported the idea that udder LY should not be impaired by low blood glucose and negative energy balance in specialized dairy cows, as mechanisms related to homeorhesis exist in specialized breeds and, thus, body reserves are used to maintain MY (Bauman and Currie, 1980; Zhao, 2014). Nevertheless, even if a low availability of blood glucose in high-yielding cows may not impair LY and MY, it could affect other physiological processes related to fertility, such as ovulation. Francisco et al. (2003) estimated a positive association between milk LP and number of days from first to second postpartum ovulation. Moreover, Buckley et al. (2003) reported favorable correlations between LP and fertility traits in Holstein cows, thus suggesting that LP could be used as indicator of reproductive performances in cattle. Miglior et al. (2006) reported a favorable correlation between LP and cows' longevity, with higher and lower risk of being culled for cows yielding milk with low and high LP, respectively. In addition, some studies proposed fat-to-lactose ratio as an indicator of metabolic status of cows (Reist et al., 2002; Ederer et al., 2014; Haile-Mariam and Pryce, 2017), as the estimated correlation with cow energy balance in the first 10 d after calving was −0.589 (Reist et al., 2002).

With respect to udder health, LP has been reported to be negatively associated with SCC (Gillon et al., 2010), the most widely used milk indicator of IMI and MAS. Moreover, LP has been recently considered a useful and reliable indicator of MAS (Ebrahimie et al., 2018; Marchitelli et al., 2018) due to its relation with MAS-related changes in milk minerals, electrical conductivity, and osmotic equilibrium. When MAS occurs, the SCC increases and the permeability of the cell membranes changes. These mechanisms lead to a drop of LP in milk and to an increase in both MAS-related minerals from blood (Na and Cl) and milk electrical conductivity (Fox et al., 2015). Nevertheless, almost all genetic and phenotypic studies that have investigated IMI and included LP and LY have dealt with SCC rather than with real diagnoses of MAS recorded by veterinarians or farmers. From a genetic point of view, because MAS is positively related to MY, genetic selection to increase the productivity in the last half century has resulted in dairy cows being more susceptible to udder inflammation (Oltenacu and Broom, 2010). Moreover, concerns exist regarding the use of SCC as a phenotype to improve MAS resistance. In fact, as SCC are mostly composed of white blood cells, one may wonder if the

selection for depressing this trait would impair immune system of the cows and thus weaken their ability to face inflammations and infections (Heringstad et al., 2000; Rainard et al., 2018). Despite these evident biological aspects relating LP and LY with cow metabolism and udder health, a proper investigation on lactose and its ratios with other milk solids have not been performed so far. The associations of LP and LY with KET, MAS, and other common metabolic and reproductive diseases of dairy cows, such as milk fever (**MFV**), retained placenta (**RET**), and ovarian cysts (**CYS**), have also not been investigated. Finally, it is worth pointing out that LP is routinely recorded in several countries in the framework of official milk recording schemes, which means that wide phenotypic information is available at the population level. Therefore, the aims of our study were to (1) estimate genetic variation and heritability of LP, its ratios with other milk solids, and LY, and (2) assess their genetic associations with major disorders in a large data set of Austrian Fleckvieh (**FV**) cows.

MATERIALS AND METHODS

Milk Data

For the observation period between January 2010 and March 2018, more than 4 million test-day records from 175,154 FV cows in 10,530 herds were retrieved from the official database of the Austrian milk recording system. The FV is the dominant cattle breed in Austria (75.3% of total registered cows) and lactation MY, fat percentage (**FP**), and protein percentage (**PP**) average 7,345 kg, 4.16%, and 3.42%, respectively (ZAR, 2018). Milk of FV cows is characterized by lower SCC (mean of 175,484 cells/mL, median of 61,000 cells/mL) than milk of other cattle breeds, such as Brown Swiss and Holstein (ZuchtData, 2017). Milk yield (kg/d) was available for each test-day record, as well as LP, FP, and PP, which were predicted through mid-infrared spectroscopy using a MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark). The SCC (cells/mL) was determined through Fossomatic FC (Foss Electric A/S) and values were retained if between 1,000 and 10,000,000 cells/mL. Test-day MY, LP, FP, or PP outside mean ± 3 standard deviations and those outside the range 5 to 400 DIM were discarded from the data set, resulting in 2,489,996 records. Finally, test-day LY (kg/d), fat yield (**FY**; kg/d), protein yield (**PY**; kg/d), and daily SCC (cells/d) were calculated by multiplying MY by LP, FP, PP, and SCC, respectively.

Considering the standard average productive life of FV in Austria (ZuchtData, 2017), observations of cows until fifth parity were kept in the data set. Parity-specific age at calving was restricted to 20 to 40 mo for first-, 32 to 58 mo for second-, 44 to 72 mo for third-, 54 to 86 mo for fourth-, and 66 to 100 mo for fifth-parity cows. This resulted in 2,471,422 test-day records.

To move from test days to lactation data, the test interval method proposed by the International Committee for Animal Recording (ICAR, 2017) was used. As most common disorders in lactating cows usually show higher incidence, additive genetic variance, and heritability in early and midlactation, the focus in the present study was on the first 150 DIM. Therefore, 150-d MY, LY, FY, PY, and SCC were calculated as

$$C_{150} = I_0T_1 + I_1 \times [(T_1 + T_2)/2] + I_2 \times [(T_2 + T_3)/2] + \ldots + I_{n-1} \times [(T_{n-1} + T_n)/2] + I_n \times T_n,$$

where C_{150} is the cumulative MY, LY, FY, PY, or SCC between 5 and 150 DIM; I_0 is the interval between 5 DIM and the first available test day; I_1, I_2, and I_{n-1} are the intervals (d) between 2 consecutive test days; I_n is the interval between the last test day and 150 DIM; and T_1, T_2, T_{n-1}, and T_n are test-day MY, LY, FY, PY, or daily SCC. The 150-d average LP, FP, and PP were computed using the following formula (ICAR, 2017):

$$AP = (C_{150}/MY_{150}) \times 100,$$

where AP is the 150-d average LP, FP, or PP; C_{150} is the 150-d LY, FY, or PY; and MY_{150} is the 150-d MY. The 150-d average SCC was calculated by dividing 150-d SCC by MY_{150}, and values were then log-transformed to SCS as $SCS = 3 + \log_2(SCC/100{,}000)$, to reach a Gaussian-like frequency distribution. Moreover, lactose-to-fat (**L:F**), lactose-to-protein (**L:P**), and lactose-to-solids (**L:S**) ratios were calculated using the 150-d average LP, FP, and PP. The final data set consisted of 284,193 lactation records of 113,722 cows from 24,326 contemporary groups, which were defined as herd-year of calving of the cow (**HY**). All cows calved between January 2010 and October 2017 and were offspring of 4,293 sires and 83,131 dams.

Health Data

Details of the monitoring of cow health in Austria can be retrieved from Fuerst et al. (2011) and Egger-Danner et al. (2012). To keep the most reliable information, diagnoses of MAS, KET, MFV, CYS, and RET were retained in the data set if collected in farms with more than 75% of diagnostic data submitted electronically by veterinarians. According to diagnoses, the observation period was between −10 and 150 DIM, except

for RET, which was diagnosed from calving to 7 DIM. Similarly to the approach of Koeck et al. (2010a,b), each disorder was treated as a binary trait (0 = absence, 1 = presence) based on whether or not the disorder was diagnosed within the time interval considered. To check the frequency of MAS, KET, MFV, CYS, and RET at different levels of milk LP, lactation data were grouped into 3 classes of LP, according to mean LP ± 2 standard deviations. The classes were created within primiparous and multiparous cows.

Genetic Parameters Estimation

A subset of 50% of the contemporary groups were randomly selected from the full data set, resulting in 142,285 lactation records from 84,289 cows, of which 14,340 had more than 2 calvings. The subset was representative of the variability of the entire data and, thus, variance and covariance components of MAS, KET, MFV, CYS, and RET, as well as of 150-d MY, LY, LP, ratios, and SCS, were estimated on the subset through univariate and bivariate analyses, respectively; this allowed to limit computational memory and time. A linear model was chosen to estimate genetic parameters of the studied traits, including health traits, which is the same approach used for routine Austrian-German genetic evaluation (Fuerst et al., 2011). In a comparative study, Koeck et al. (2010a,b) reported that linear models were reliable tools for genetic analysis of health traits. The general form of the mixed linear animal model was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wp} + \mathbf{Vq} + \mathbf{e},$$

where $\mathbf{y}$ is the vector of phenotypic records of the trait; $\mathbf{b}$ is the vector of fixed effects of parity (from first to fifth), age at calving within parity (3 classes for first-parity cows: <28, 28–30, and >30 mo; 3 classes for second-parity cows: <41, 41–44, and >44 mo; 3 classes for third-parity cows: <53, 53–57, and >57 mo; and 1 class for fourth- and fifth-parity cows), and month-year of calving (94 levels); $\mathbf{a}$ is the vector of random animal additive genetic effects; $\mathbf{p}$ is the vector of random permanent environmental effects; $\mathbf{q}$ is the vector of random HY effects; $\mathbf{e}$ is the vector of random residuals; and $\mathbf{X}$, $\mathbf{Z}$, $\mathbf{W}$, and $\mathbf{V}$ are incidence matrices relating the corresponding effects to the dependent variable. All the analyses were performed using ASReml 4.1 (Gilmour et al., 2015).

Heritability was calculated as ratio of additive genetic to phenotypic variance (i.e., sum of additive genetic, permanent environmental, HY, and residual variances) and repeatability was the ratio between sum of additive genetic and permanent environmental variances to phenotypic variance. Genetic ($\mathbf{r_a}$) and phenotypic ($\mathbf{r_p}$) correlations were computed as

$$r = \frac{cov_{1,2}}{\sqrt{\sigma_1^2 \times \sigma_2^2}},$$

where $cov_{1,2}$ is the additive genetic or phenotypic covariance between trait 1 and trait 2; σ_1^2 is the additive genetic or phenotypic variance of trait 1; and σ_2^2 is the additive genetic or phenotypic variance of trait 2.

The reliability of the pedigree provided by ZuchtData (Vienna, Austria) was checked with the software CFC 1.0 (Sargolzaei et al., 2006), and more than half of the individuals had coefficients of inbreeding between 0 and 5%. Ancestors of target cows were traced back 4 generations, which resulted in 195,356 individuals for the subset.

RESULTS AND DISCUSSION

Descriptive Statistics and Pearson's Correlations

Descriptive statistics calculated on the full data set (n = 284,193) showed that 150-d MY averaged 4,157 kg (Table 1), which corresponds to an average daily MY of 27.7 kg. Averages of FP and PP were 4.03 and 3.28%, respectively. Means of FP and PP were expected to be lower than those of the whole lactation because the present study focused on the first 150 DIM. Indeed, ZAR (2018) reported average FP and PP of 4.16 and 3.42%, respectively, and Fuerst-Waltl et al. (2016) reported FP of 4.12% and PP of 3.45% for first-lactation FV cows. For the same breed, Ederer et al. (2014) reported a milk composition in early lactation similar to that of the present study. The average LP (4.83%) was close to the value (4.84%) assessed by Ederer et al. (2014); however, comparisons with the literature are difficult due to paucity of studies on FV focusing on early and midlactation and including LP in the list of investigated milk traits. Some authors evaluated the effect of breed on milk composition and also estimated least squares means of LP for FV or Simmental cows. Overall, in those studies, milk LP of Simmental was 4.75% (Kedzierska-Matysek et al., 2011) and 4.71% (Gottardo et al., 2017), and it was also significantly lower than milk LP of Holstein-Friesian ($P < 0.01$; Kedzierska-Matysek et al., 2011). The L:F, L:P, and L:S averaged 1.21, 1.48, and 0.40, respectively, and their coefficients of variation ranged from 5.25 (L:S) to 12.19% (L:F). Somatic cell score was low (1.77 units) if compared with findings obtained for the whole lactation

Table 1. Descriptive statistics of and Pearson correlations ($P < 0.0001$) between 150-d yield and composition traits, ratios,[1] and SCS for the whole data set (n = 284,193)

Item	Yield, kg				Percentage			Ratio[1]			
	Milk	Lactose	Fat	Protein	Lactose	Fat	Protein	L:F	L:P	L:S	SCS
Mean	4,157	200.6	167.5	136.4	4.83	4.03	3.28	1.21	1.48	0.40	1.77
CV, %	23.06	23.03	24.88	24.51	2.63	11.73	7.85	12.19	8.27	5.25	83.31
Correlation											
Milk yield, kg		0.993	0.879	0.949	−0.066	−0.100	0.027	0.084	−0.060	0.040	−0.064
Lactose yield, kg			0.866	0.943	0.047	−0.108	0.026	0.115	−0.024	0.080	−0.099
Fat yield, kg				0.876	−0.093	0.379	0.162	−0.379	−0.192	−0.373	−0.037
Protein yield, kg					−0.063	−0.001	0.332	−0.010	−0.345	−0.154	−0.048
Lactose, %						−0.066	−0.012	0.268	0.324	0.350	−0.313
Fat, %							0.305	−0.963	−0.302	−0.875	0.047
Protein, %								−0.291	−0.943	−0.627	0.047
L:F									0.354	0.912	−0.113
L:P										0.698	−0.141
L:S											−0.148

[1]L:F = lactose-to-fat ratio; L:P = lactose-to-protein ratio; L:S = lactose-to-solids ratio.

of FV and other breeds, but similar to the value (1.90) reported by Koeck et al. (2010b) for the same breed in a similar observation period (8–100 DIM). Indeed, milk SCC is lower in early than late lactation both for physiological reasons and because of the dilution effect.

Overall, the strongest Pearson correlation (0.993) was assessed between MY and LY (Table 1). This estimate was similar to the value (0.99) reported by Sneddon et al. (2015) in a New Zealand multibreed study and confirmed the strong physiological relationship between the 2 traits. Lactose yield weakly correlated with all traits, and LP was moderately associated with ratios (from 0.268 with L:F to 0.350 with L:S). Lactose-to-fat ratio and L:P were moderately associated (0.354), and correlations of L:S with L:F and L:P were strong (0.912 and 0.698, respectively). Moreover, ratios were negatively weakly associated with SCS.

Frequency of Health Disorders

Contrary to the trend of LP, the incidence of diagnosed cows for health disorders increased with parity order (Table 2); this suggests that older cows yielded milk with lower LP and were more prone to get sick than younger animals. In particular, for MFV, the frequency increased considerably from 0.20 (first-parity cows) to 6.00% (fifth-parity cows); these distributions were similar to those reported by Egger-Danner et al. (2012) in Austria. Moreover, Ederer et al. (2014) assessed frequencies of KET and MFV across parities that were 0.60 and 2.80%, respectively. In studies including other popular dairy breeds, such as Holstein, the incidence of KET was usually higher (4.10%; Koeck et al., 2013). The incidence of CYS was slightly lower than that (7.70%) reported by Hooijer et al. (2001) in Dutch Black and White cows.

As expected, moving from the low-LP to the high-LP class, SCS decreased in both primiparous and multiparous cows. In particular, SCS was highest (3.538 ± 1.764) in the low-LP class in multiparous cows (Table 3), whereas the lowest SCS (1.014 ± 1.130) was assessed in the high-LP class of primiparous cows. Overall, the incidence of CYS slightly decreased from high to low LP. With regard to MFV, primiparous were less susceptible than multiparous animals; in fact, the greatest incidence (3.76%) was found in the low-LP class of multiparous cows. The incidence of RET was slightly higher in the low-LP class compared with medium- and high-LP classes. In all other cases, the frequencies increased from high to low LP. In fact, the frequencies of MAS, KET, and RET of the low-LP class were 1.33, 1.41, and 1.02 times those of the high-LP class in primiparous animals, respectively. Considering multiparous cows, the frequencies of MAS, KET, MFV, and

Table 2. Parity-specific number of observations (n), average lactose percentage, and frequency (%) of health disorders[1] for the data set (n = 284,193) and subset[2] (n = 142,285)

Parity	n		Lactose percentage[3]		Mastitis		Ketosis		Milk fever		Ovarian cysts		Retained placenta	
	Data set	Subset	Data set	Subset	Data set	Subset	Data set	Subset	Data set	Subset	Data set	Subset	Data set	Subset
1	87,566	44,059	4.89	4.89	4.60	4.61	0.62	0.65	0.20	0.20	4.38	4.43	1.08	1.08
2	71,078	35,434	4.82	4.82	5.12	5.13	0.49	0.49	0.76	0.79	6.27	6.43	1.74	1.77
3	55,757	27,897	4.80	4.80	6.18	6.18	0.81	0.75	2.21	2.14	7.20	7.11	2.09	2.09
4	41,382	20,746	4.78	4.78	7.88	7.87	0.93	0.91	4.54	4.43	7.75	7.93	2.23	2.14
5	28,410	14,149	4.77	4.77	8.61	8.63	1.00	1.05	6.00	6.04	8.10	8.38	2.37	2.46

[1]Coded as binary traits (absence = 0, presence = 1) for the observation period −10 to 150 DIM, except for retained placenta (from calving to 7 DIM).
[2]Average values of the subset within parity were not statistically different from those of the data set ($P > 0.05$) after 1-sample *t*-test and goodness-of-fit test for lactose percentage and health traits frequencies, respectively.
[3]SD were within the range 0.11 to 0.13.

RET of the low-LP class were 2.33, 1.27, 1.83, and 1.17 times those of the high-LP class, respectively. Regarding CYS, its incidence somehow decreased moving from high to low LP in both primiparous and multiparous cows, suggesting that, for some reasons, cows with high LP were more susceptible to CYS. Overall, frequencies of diagnosed disorders in first-lactation cows were lower than those detected in cows in subsequent lactations. In particular, first-parity cows were at much lower risk of developing MFV at calving, likely because they did not suffer from metabolic stress caused by both previous lactation and dry period. Comparing the incidence of health disorders within classes of LP, fewer differences for first-lactation cows compared with other lactations were generally detected (Table 3).

Variance Components and Heritability

Heritabilities of the investigated traits ranged from 0.005 ± 0.001 (RET) to 0.566 ± 0.008 (LP; Table 4). Similar estimates for LP were reported by Miglior et al. (2007) for Canadian Holsteins and by Stoop et al. (2007) for first-calving Holstein cows in the Netherlands using test-day data. Lactose yield was the least heritable (0.145 ± 0.005) among milk-related traits, but this estimate was comparable with heritabilities of 0.125 and 0.180 reported by Tiezzi et al. (2013) in Italy and Sneddon et al. (2015) in New Zealand, respectively. Moreover, this value was similar to that (0.140) reported by Costa et al. (2018) in Italian Holstein. Ratios were moderately heritable, with estimates from 0.445 ± 0.008 (L:F) to 0.538 ± 0.008 (L:S), but it was not possible to compare our estimates with the literature due to absence of studies including the same traits. Mastitis, KET, MFV, CYS, and RET were lowly heritable and estimates were similar to those reported by Pryce et al. (2016) and Egger-Danner et al. (2015), who reviewed genetic parameters of most common disorders of dairy cows. In particular, heritability of MAS in the literature ranges from 0.02 to 0.08, with differences due to breed or statistical approach (Egger-Danner et al., 2015). Focusing on the period from 51 to 150 DIM, Koeck et al. (2010b) estimated heritability of 0.02 for MAS in FV cows. Heritability of KET in the present study was lower than estimates (0.01 to 0.08) retrieved from the literature and assessed using linear models (Pryce et al., 2016). Regarding MFV, studies dealing with linear models reported an average heritability of 0.03 (Pryce et al., 2016), which is higher than the value computed in the present study. Ederer et al. (2014) reported heritability of 0.034 for MFV in a data set of FV cows. Fertility disorders were lowly heritable as well (Table 4). Development of CYS (0.037 ± 0.004) was the most heritable health trait, and the estimate was

Table 3. Mean (SD) of SCS and frequency (%) of health disorders[1] across classes of lactose percentage[2] in primiparous and multiparous cows

Class	Lactose percentage range	N	SCS	Mastitis	Ketosis	Milk fever	Ovarian cysts	Retained placenta
Primiparous								
Low	4.200–4.664	2,402	2.454[a] (1.564)	6.70[a]	0.83[a]	0.25[b]	3.46[b]	1.25[a]
Medium	4.665–5.116	83,459	1.512[b] (1.261)	4.53[b]	0.62[a]	0.19[b]	4.40[a]	1.08[a]
High	5.117–5.300	1,705	1.014[c] (1.130)	5.04[b]	0.59[a]	0.29[a]	4.69[a]	1.23[a]
Multiparous								
Low	4.180–4.553	5,400	3.538[a] (1.764)	11.52[a]	0.93[a]	3.76[a]	5.78[c]	2.56[a]
Medium	4.554–5.044	187,366	1.848[b] (1.510)	6.39[b]	0.74[a]	2.71[b]	7.08[b]	2.01[ab]
High	5.045–5.345	3,861	1.084[c] (1.269)	4.95[c]	0.73[a]	2.05[b]	10.52[a]	2.18[b]

[a–c]Values with different superscripts within trait and parity group were significantly different in multiple comparisons after Bonferroni-Holm step-down adjustment ($P < 0.05$).

[1]Coded as binary traits (absence = 0, presence = 1) for the observation period −10 to 150 DIM, except for retained placenta (from calving to DIM 7).

[2]Classes of lactose percentage were defined according to mean ± 2 SD.

almost equal to the value (0.039) estimated by Koeck et al. (2010c) in the same breed using a linear model, but lower than the heritability (0.102) assessed by Hooijer et al. (2001) in a smaller data set of 15,562 Dutch Black and White cows. Heritability of RET (0.005 ± 0.001) was also similar to the estimate (0.007) reported by Koeck et al. (2010c) for Austrian FV.

Differences with other studies could be due to (1) breed, (2) effects considered in the model, and (3) criteria adopted for recording health data. In addition, nonlinear models generally tend to slightly overestimate additive genetic variance (Heringstad et al., 2000); in fact, Heringstad et al. (2005) estimated higher heritabilities for KET (0.14 to 0.16) and MFV (0.09 to 0.13) in Norwegian Red cows using a threshold model. Moreover, with the same model, Neuenschwander et al. (2012) estimated greater heritability for KET (0.09) in Holsteins. Similarly, the adoption of probit and logit models resulted in higher heritabilities of MAS (0.06 to 0.08) in FV cows (Koeck et al., 2010a). Overall, heritabilities of fertility disorders were in agreement with the literature; in particular, Koeck et al. (2010c) reported heritabilities of 0.060, 0.006, and 0.007 for RET using logit threshold sire, linear sire, and linear animal models, respectively, in FV cows. Comparably, CYS was found more heritable with threshold model, with estimates ranging from 0.050 (Zwald et al., 2004) to 0.077 (Koeck et al., 2010c), if compared with the present and other studies that adopted linear models to analyze data (0.040; Koeck et al., 2010c).

Because we considered 150-d traits in the present study, the repeatability of milk-related traits is the sim-

Table 4. Phenotypic variance[1] $\left(\sigma_p^2\right)$, heritability (SE), repeatability (SE), and proportion (%) of variance explained by herd-year of calving effect (c^2) for 150-d production, SCS, and health traits for the subset (n = 142,285)

Trait	σ_p^2	Heritability	Repeatability	c^2
Production traits[2]				
Milk yield, kg	705,970.2	0.151 (0.005)	0.213 (0.003)	50.37
Lactose yield, kg	1,678.98	0.145 (0.005)	0.204 (0.003)	50.73
Lactose, %	*13.97*	0.566 (0.008)	0.624 (0.003)	6.16
L:F	*21.82*	0.445 (0.008)	0.497 (0.003)	9.22
L:P	*13.14*	0.474 (0.008)	0.507 (0.003)	12.52
L:S	*0.42*	0.538 (0.008)	0.574 (0.003)	7.26
SCS	2.12	0.183 (0.007)	0.308 (0.004)	10.02
Health traits[3]				
Mastitis	*55.61*	0.017 (0.003)	0.050 (0.004)	6.11
Ketosis	*7.00*	0.006 (0.002)	0.033 (0.004)	3.56
Milk fever	*18.45*	0.015 (0.002)	0.042 (0.004)	2.63
Ovarian cysts	*58.75*	0.037 (0.004)	0.071 (0.004)	10.55
Retained placenta	*17.07*	0.005 (0.001)	0.027 (0.004)	2.74

[1]Values in italics have to be multiplied by 10^{-3}.

[2]L:F = lactose-to-fat ratio; L:P = lactose-to-protein ratio; L:S = lactose-to-solids ratio.

[3]Coded as binary traits (absence = 0, presence = 1) for the observation period −10 to 150 DIM, except for retained placenta (from calving to 7 DIM).

ilarity of values of the trait on the same cow in different lactations. Therefore, comparisons of repeatabilities of MY, LY, LP, and ratios with those estimated with test-day repeatability models are not fully correct. Both LP and ratios had moderate to high repeatabilities (from 0.497 ± 0.003 of L:F to 0.624 ± 0.003 of LP). Even with different statistical approaches and designs, similar findings for LP were reported by Stoop et al. (2007), Sneddon et al. (2015), and Visentin et al. (2017). The low repeatability for LY suggested that the synthesis of this component in the udder differed across lactations of the same cow in the first 150 DIM. Somatic cell score exhibited a moderate repeatability (0.308 ± 0.004), slightly lower than that reported by Tiezzi et al. (2013) and Visentin et al. (2017). Health traits, on the other hand, were scarcely repeatable, with values that ranged from 0.027 ± 0.004 (RET) to 0.071 ± 0.004 (CYS); the low repeatability of health traits was expected, as they are supposed to be occasional events and their frequency across lactations are usually close to zero, due to culling, therapy, and other management strategies.

The greatest contribution of the HY effect to the phenotypic variance was found for LY (50.73%), suggesting that temporary effects of HY are important in determining the variance of this trait. Similarly, the HY effect accounted for 50.37% of MY variance. With regard to other milk traits, the proportion of phenotypic variance explained by the HY effect varied from 6.16% (LP) to 12.52% (L:P). Finally, the HY effect was 6.11, 3.56, 2.63, 10.55, and 2.74% for MAS, KET, MFV, CYS, and RET, respectively, which suggested that the environmental HY effect had a moderate to low effect on the phenotypic variance of the health traits.

Phenotypic and Genetic Correlations

All r_p between health and production traits were very weak, with the greatest association estimated between LY and CYS ($r_p = 0.074$; Table 5), highlighting that, at phenotypic level, no association exists between lactose and its ratios with health traits. Mastitis was unfavorably genetically correlated with LY (0.518 ± 0.057), favorably and weakly associated with LP (−0.175 ± 0.049), and almost uncorrelated with L:F, L:P, and L:S (Table 5). In addition, MAS was positively genetically correlated with SCS ($r_a = 0.623 \pm 0.056$ and $r_p = 0.158 \pm 0.060$) and LP was negatively related to SCS ($r_a = -0.245 \pm 0.018$ and $r_p = -0.288 \pm 0.003$) and MY ($r_a = -0.175 \pm 0.018$) in the first 150 DIM. These findings were similar to results of Bastin et al. (2016), who reported r_a between LP and MAS of −0.10 and −0.24 for the observation periods 5 to 305 and 5 to 65 DIM, respectively. Lactose yield was positively genetically correlated with KET (0.420 ± 0.077), MFV (0.219 ±

Table 5. Genetic (r_a) and phenotypic (r_p) correlations (SE within parentheses) between health traits[1] and between health and production traits estimated on the subset (n = 142,285)

	Mastitis		Ketosis		Milk fever		Ovarian cysts		Retained placenta	
Trait[2]	r_a	r_p	r_a	r_p	r_a	r_p	r_a	r_p	r_a	r_p
Lactose yield	0.518 (0.057)	0.006 (0.003)	0.420 (0.077)	0.015 (0.003)	0.219 (0.063)	0.040 (0.003)	0.189 (0.043)	0.074 (0.004)	0.000 (0.000)	−0.015 (0.003)
Lactose percentage	−0.175 (0.049)	−0.041 (0.003)	−0.158 (0.074)	−0.012 (0.003)	−0.094 (0.050)	−0.006 (0.003)	0.071 (0.035)	0.033 (0.003)	−0.037 (0.079)	−0.001 (0.003)
L:F	−0.013 (0.052)	−0.012 (0.003)	0.077 (0.080)	−0.011 (0.003)	0.054 (0.054)	−0.002 (0.003)	−0.084 (0.038)	−0.020 (0.003)	−0.038 (0.084)	0.000 (0.003)
L:P	0.073 (0.051)	−0.028 (0.003)	0.198 (0.078)	0.022 (0.003)	0.098 (0.053)	0.008 (0.003)	0.036 (0.037)	0.016 (0.003)	−0.056 (0.084)	−0.018 (0.003)
L:S	−0.008 (0.050)	−0.020 (0.003)	0.056 (0.053)	0.003 (0.003)	0.001 (0.051)	0.004 (0.003)	−0.001 (0.012)	0.000 (0.003)	−0.041 (0.082)	−0.008 (0.003)
Mastitis	—	—	0.355 (0.135)	0.026 (0.003)	0.463 (0.090)	0.044 (0.003)	0.084 (0.089)	0.034 (0.003)	0.000 (0.000)	0.028 (0.003)
Ketosis			—	—	0.462 (0.119)	0.067 (0.003)	0.059 (0.119)	0.027 (0.003)	0.286 (0.172)	0.019 (0.003)
Milk fever					—	—	0.273 (0.087)	0.034 (0.003)	0.084 (0.146)	0.023 (0.003)
Ovarian cysts							—	—	0.000 (0.000)	0.017 (0.003)

[1]Coded as binary traits (absence = 0, presence = 1) for the observation period −10 to 150 DIM, except for retained placenta (from calving to 7 DIM).
[2]L:F = lactose-to-fat ratio; L:P = lactose-to-protein ratio; L:S = lactose-to-solids ratio.

0.063), and CYS (0.189 ± 0.043). In addition, KET was genetically associated with L:P (0.198 ± 0.078) and LP (−0.158 ± 0.074). All other r_a of LY, LP, and ratios with health traits were <0.10 (Table 5).

Phenotypic correlations between health traits were almost zero, with the greatest estimate between KET and MFV (0.067). At the genetic level, MAS positively related to other diseases typical of early lactation [i.e., KET (0.355 ± 0.135) and MFV (0.463 ± 0.090)]. Both correlations were in the ranges of previous studies (Pryce et al., 2016) and suggested that selection against MAS leads to favorable correlated responses in other diseases (Pfeiffer et al., 2015). Supporting this view, Pryce et al. (2016) suggested that a general cow's robustness is responsible for its susceptibility to get sick. The r_a of CYS with MAS, KET, and MFV were positive, suggesting that partly there could be common genetic background between these traits; in particular, the greatest r_a (0.273) was estimated between CYS and MFV. Considering that the r_p between CYS and MFV was almost zero, these findings suggested that genetically MFV could predispose cows to CYS during the first 150 DIM and that daughters of bulls with high EBV for MFV are supposed to be at higher risk of developing CYS compared with offspring of bulls with lower EBV for MFV. This could be related to the altered mineral metabolism in presence of MFV that could impair the hormonal regulation. In addition, it is fair to state that cow susceptibility also depends on the interaction between genotype and environment; this could explain the higher r_a between MAS and CYS (0.16) reported in an earlier study on the same breed (Pfeiffer et al., 2015). Finally, RET was genetically (0.286 ± 0.172) associated with KET (Table 5).

All correlations estimated in the present study were in the ranges reported by Ingvartsen et al. (2003), who reviewed genetic associations between milk performance and cow health, and by Van Dorp et al. (1998), who estimated the correlation between 305-d MY and health disorders in Holstein cows. These findings confirmed that the genetic background of high-producing cows makes them more susceptible to diseases than that of cows yielding less milk (Ingvartsen et al., 2003; Oltenacu and Broom, 2010) and confirmed that LP is favorably genetically related to cows' energy balance and udder health (Bastin et al., 2016).

CONCLUSIONS

Cows with lower milk LP had higher frequency of udder health and metabolic disorders. The amount of LP variance due to additive genetic effects was >50% and the genetic correlation with MAS in the first 150 DIM was negative. Genetic and phenotypic correlations of LY almost mirrored those of MY. Ratios including LP and other milk composition traits did not genetically correlate with health traits, except for the weak positive genetic relationship between L:P and KET. Based on the findings of the present study, LP is a valid candidate as an udder health indicator in early lactation. In fact, high heritability coupled with great data availability make LP a potential phenotype to be considered in the FV breeding program for mastitis resistance and udder health, thus far including lowly to moderately heritable traits such as MAS, SCC, and udder conformation traits.

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Chapter IV

On the genomic regions associated with milk lactose in Fleckvieh cattle

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On the genomic regions associated with milk lactose in Fleckvieh cattle

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ABSTRACT

Lactose is a sugar uniquely found in mammals' milk and it is the major milk solid in bovines. Lactose yield (LY, kg/d) is responsible for milk volume, whereas lactose percentage (LP) is thought to be more related to epithelial integrity and thus to udder health. There is a paucity of studies that have investigated lactose at the genomic level in dairy cows. This paper aimed to improve our knowledge on LP and LY, providing new insights into the significant genomic regions affecting these traits. A genome-wide association study for LP and LY was carried out in Fleckvieh cattle by using bulls' deregressed estimated breeding values of first lactation as pseudo-phenotypes. Heritabilities of first-lactation test-day LP and LY estimated using linear animal models were 0.38 and 0.25, respectively. A total of 2,854 bulls genotyped with a 54K SNP chip were available for the genome-wide association study; a linear mixed model approach was adopted for the analysis. The significant SNP of LP were scattered across the whole genome, with signals on chromosomes 1, 2, 3, 7, 12, 16, 18, 19, 20, 28, and 29; the top 4 significant SNP explained 4.90% of the LP genetic variance. The signals were mostly in regions or genes with involvement in molecular intra- or extracellular transport; for example, *CDH5*, *RASGEF1C*, *ABCA6*, and *SLC35F3*. A significant region within chromosome 20 was previously shown to affect mastitis or somatic cell score in cattle. As regards LY, the significant SNP were concentrated in fewer regions (chromosomes 6 and 14), related to mastitis/somatic cell score, immune response, and transport mechanisms. The 5 most significant SNP for LY explained 8.45% of genetic variance and more than one-quarter of this value has to be attributed to the variant within *ADGRB1*. Significant peaks in target regions remained even after adjustment for the 2 most significant variants previously detected on BTA6 and BTA14. The present study is a prelude for deeper investigations into the biological role of lactose for milk secretion and volume determination, stressing the connection with genes regulating intra- or extracellular trafficking and immune and inflammatory responses in dairy cows. Also, these results improve the knowledge on the relationship between lactose and udder health; they support the idea that LP and its derived traits are potential candidates as indicators of udder health in breeding programs aimed to enhance cows' resistance to mastitis.

Key words: lactose, genome-wide association study (GWAS), inflammatory response, molecular transport, bovine milk

INTRODUCTION

Lactose is the natural sugar present in milk of mammals and its concentration is predicted using mid-infrared spectroscopy in individual and bulk milk samples during routine recording schemes. Lactose percentage (**LP**) is associated with udder health in cattle (Ebrahimie et al., 2018; Costa et al., 2019a); in fact, as soon as IMI and inflammation occur, milk LP decreases. Overall, moderate correlations between LP and SCS, the most adopted indicator of IMI worldwide, have been reported in the literature, with peaks of −0.44 (Stoop et al., 2007) and −0.66 (Vilas Boas et al., 2017) for genetic and phenotypic correlations, respectively. Moreover, Costa et al. (2019a) estimated a negative genetic correlation (−0.18) between LP and mastitis in Fleckvieh (**FV**) cows in early to mid lactation. In the presence of IMI, the permeability of epithelial cells changes and lactose is partly lost in the bloodstream (Bansal et al., 2005); this explains why plasma LP is an indicator of epithelial integrity in cattle (Herve et al., 2019). As regards lactose yield (**LY**), this trait is closely related to milk volume, because it is the major milk osmole; in

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fact, the amount of synthesized lactose directly determines water uptake in the alveoli (Fox et al., 2015). This means that the higher the LY upstream, the higher the milk yield. The ability to synthesize a high amount of lactose might be related to greater expression of genes encoding glucose transporters, which are responsible for glucose uptake from blood (Zhao, 2014), and also to greater blood sugar availability. The mammary gland is the first target tissue for the delivery of substrates as glucose; this supports the fact that the metabolic priority of dairy cows is milk synthesis and that the udder is subjected to homeorhesis (Bauman and Currie, 1980). Moreover, the Golgi is the cellular environment specialized for lactose synthesis, and greater genetic expression of its enzymes in mammary tissue could further affect LP, LY, or both. Despite this, little is known about the physiological and biological paths behind conversion of glucose to lactose in the mammary gland and the association of LP with mastitis or IMI. In fact, interest in this milk component has only recently increased in the scientific community (Sneddon et al., 2015, 2016; Haile-Mariam and Pryce, 2017; Costa et al., 2018, 2019b). Milk yield, and fat and protein content and yield are usually included in the most common routine genetic or genomic evaluation schemes in Europe and are thus the target phenotypes of genome-wide association studies (**GWAS**) in dairy cattle (Wang and Bovenhuis, 2018). Conversely, only a few GWAS for LP and LY have been performed in cattle (Lopdell et al., 2017; Wang and Bovenhuis, 2018). Thus, an opportunity exists to investigate genomic regions that affect LP and LY to better understand which genes influence these traits.

The FV breed has a share of about 75% of Austrian controlled dairy cows (ZAR, 2018), and a joint genetic evaluation is carried out for FV by Austria, Germany, and the Czech Republic. This cooperative system allows generation of both big data and accurate genomic EBV, whose routine estimation was recognized by the International Committee for Animal Recording in 2011. Although LP and LY have recently been reported to be genetically associated with mastitis and ketosis in FV (Costa et al., 2019a), information on genomic regions affecting LP and LY is lacking for this breed. Therefore, in this study, we aimed to perform a GWAS to better understand the genetic background of LP and LY, and to evaluate accordance with genomic regions affecting udder health traits, such as mastitis, IMI, and SCS.

MATERIALS AND METHODS

Genotypes

Genomic data of 7,003 purebred FV bulls were jointly provided by Austria, Germany, and the Czech Republic. All individuals were genotyped with the 54K Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA).

Pseudo-Phenotypes

The bulls' deregressed EBV of first lactation were used as pseudo-phenotypes in the GWAS. First, variance components and heritability of LP and LY (Table 1) were estimated using the VCE6 software, version 6.0 (Neumaier and Groeneveld, 1998). For this purpose, 198,038 test-day milk yield and infrared-predicted LP records of 54,878 cows representing a subset of the entire data (>16 million milk test-day records and >1 million cows) were used; LY was calculated as (LP/100 × milk yield). The single-trait test-day repeatability animal model included the fixed effects of region-year-month of sampling, herd-year-season of sampling, age at calving, and DIM (linear and quadratic covariate), and the random effects of permanent environment, additive genetic animal, and residual. Bulls' EBV for first lactation were then estimated using the MiX99 software (Lidauer et al., 2015) by applying the above-mentioned model on all test-day records with reliable information on LP and LY (n = 7,065,937; Table 1). The pedigree included 1,824,262 animals; that is, cows with phenotypic observations and all available generations of ancestors. A deregression was carried out based on the approach proposed by Jairath et al. (1998) and Schaeffer (2001), implemented in the software MiX99 (Lidauer et al., 2015). Finally, only bulls with an estimated daughter contribution ≥10 were considered for further investigation, which led to 3,566 and 3,558 bulls for LP and LY, respectively.

Table 1. Descriptive statistics (7,065,937 test-day records) and heritability[1] of lactose percentage and lactose yield in first-parity cows

Trait	Mean	CV (%)	Minimum	Maximum	Heritability (SE)
Lactose percentage (%)	4.86	3.29	4.00	5.49	0.38 (0.01)
Lactose yield (kg/d)	0.98	28.57	0.12	3.74	0.25 (0.01)

[1]Estimated using a subset of 198,038 test-day records.

Quality Control

The quality control was performed with the software PLINK 1.9 (Purcell et al., 2007; Chang et al., 2015). The quality of SNP was ensured by removing variants with call rate <0.90, minor allele frequency <1%, and deviating significantly (P-value <1.00E-6) from Hardy-Weinberg equilibrium. A call rate ≥0.90 was required for bulls to be included. After editing, 40,486 SNP and 2,854 bulls for LP, and 40,094 SNP and 2,853 bulls for LY were available for the GWAS.

Association Study

The linear mixed model approach implemented in GEMMA software (Zhou and Stephens, 2012) for the GWAS was

$$\mathbf{y} = \mu + \mathbf{X}\beta + \mathbf{u} + \boldsymbol{\varepsilon},$$

where $\mathbf{y}$ is the vector of phenotypes (deregressed EBV of LP or LY); μ is the intercept; $\mathbf{X}$ is a vector of marker genotypes; β is the effect size of the markers; $\mathbf{u}$ is a vector of random individual effects; and $\boldsymbol{\varepsilon}$ is a vector of errors. Log-likelihood ratio statistics were adopted to test the null hypothesis that a polymorphism did not affect the phenotype; that is, H_0: $\beta = 0$ was tested against H_1: $\beta \neq 0$ for each SNP. In addition, the genomic relationship matrix was included in the analysis to account for population structure and avoid the presence of systematic bias. Because the Bonferroni correction is usually too restrictive and does not account for the fact that several SNP may trace the same QTL because of linkage disequilibrium (Goddard and Hayes, 2012), the false discovery rate with a cut-off at 0.20 (P-value <0.00013) was used as criterion to fulfill the P-value thresholds for significance discrimination (Efron, 2007). Graphical representations of Manhattan and quantile-quantile plots were obtained in the R software using the "CMplot" (Yin, 2016) and "qqman" packages (Turner, 2018), respectively. The proportion of genetic variance explained by a target SNP (**SNP-t**) and its variants in linkage disequilibrium was derived in the GCTA software (Yang et al., 2011). In particular, genetic variance was estimated using 2 different genomic relationship matrices: one for the SNP-t (with variants in linkage disequilibrium) and one for the remaining SNP. Then, the proportion of variance explained by the SNP-t was derived as the ratio between the 2 variances. Finally, significant genes were identified on the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov/; NCBI, 2018) by mapping polymorphisms referring to the genome assembly ARS-UCD 1.2. "Nearby" genes were defined to be at most ±0.1 Mb distant from the significant SNP.

RESULTS AND DISCUSSION

Lactose Percentage

The significant variants detected in the GWAS for LP are listed in Table 2 and the quantile-quantile plot is shown in Figure 1. Within the genome, signals of LP were spread across several chromosomes (Figure 2), which confirms that LP is a polygenic trait controlled by multiple regions with cumulative effects (Goddard et al., 2016). The amount of genetic variance explained by the 4 most significant SNP was 4.90%, of which one-third was attributed to ARS-BFGL-NGS-39978 (P = 4.132E-08) that is close to (≤0.1 Mb) *NEMP2*, *NAB1*, *MFSD6*, and *LOC104971101* (BTA2). Table 3 provides an overview of genes detected and Table 4 summarizes their known major functions. Several polymorphisms were in mastitis and inflammation response-related regions; in particular, a variant (154.087 Mb, BTA1) was detected within the gene *PLCL2*, which includes leukocyte B proliferation and immune response regulation among its functions. Additionally, a significant SNP (15.526 Mb, BTA3) was detected near *EFNA1*, which encodes ephrin, a protein related to the inflammatory response in mammary cells and the integrity of mammary epithelial cells (Kang et al., 2018). The gene *ANKH* in BTA20 was close to 2 significant variants previously detected by Tiezzi et al. (2015) in a GWAS for mastitis in first-parity US Holsteins. Similarly, Meredith et al. (2013) found variants within the window 57.65 to 57.75 Mb of BTA20 that affected SCS in Holstein cows in Ireland. This was confirmed by Lopdell et al. (2017), who reported a significant signal for LP at 58.45 Mb (BTA20). A significant polymorphism in BTA1 was found within *PAK2*, regulator of signal transduction, and near *NRROS*, involved in immune response and oxidative activity. Significant signals, including that with the lowest P-value, were in the region from 5.75 to 6.70 Mb of BTA2, known to be related to transmembrane transport activity and including *MFSD6*, *NEMP2*, and *SLC40A1*. In particular, among the functions of *MFSD6*, it is worth mentioning the roles in reception of macrophages and facilitation of intra- or extracellular transport (NCBI, 2018). A SNP on BTA7 was within *RASGEF1C*, regulator of membrane-associated activity and vesicle trafficking. The *CDH5* gene, regulator of cell polarity, was detected on BTA18, whereas the membrane-associated protein *ABCA6* and the junction protein *GJC1* were within BTA19. Among all functions of the *ABCA* gene family, it is worth mentioning the

molecular transport within and among cells, as for *RASGEF1C* (BTA7) and *SLC35F3* (BTA28); moreover, *ABCA6* is related to leukocytes and antimicrobial activity (Wathes et al., 2019) and it has been found significant for mastitis in Holstein cows, together with its neighbors (*ABCA5*, *ABCA9*, and *ABCA10*; Tiezzi et al., 2015). The *KCNK1* gene was close to 2 signals on BTA28; it codes for proteins involved in potassium and sodium channel activities and for stabilization of membrane potential in epithelial cells. In addition, *KCNK1* is well expressed in mammary tissue and is related to mastitis in mice (Ogorevc et al., 2009). A couple of significant SNP on BTA20 were near *ANKH*, a mastitis-related gene in Holsteins (Tiezzi et al., 2015). Finally, a few variants were within genes without functions related to either IMI or transport mechanisms: *LOC112449084* and *LOC784305* (BTA12), *LOC101904639* (BTA16), and *LOC112442737* (BTA19). These signals were also detected for LP by Lopdell et al. (2017) in Holstein and Jersey cows in New Zealand. The region within BTA19 (33.51 to 61.13 Mb), known to affect LP (Lopdell et al., 2017; Wang and Bovenhuis, 2018), was here picked up by 3 significant SNP, 2 of them located at 61.017 and 61.441 Mb, which were also neighboring the SNP (58.45 Mb) detected by Lopdell et al. (2017). The effect of BTA28 region (at around 6.56 Mb) was confirmed in the present study by the presence of 3 significant variants (6.55 to 6.89 Mb). The SNP on BTA29 (9.32 Mb) was not far from the one (9.61 Mb) detected by Lopdell et al. (2017), and Wang and Bovenhuis (2018). Finally, no signals were detected at 8.70 Mb of BTA28 or 37.1 Mb of BTA6, both thought to significantly affect LP (Lopdell et al., 2017; Wang and Bovenhuis, 2018).

These findings make LP an interesting trait for genetic purposes in cattle and may be used to better understand causality between IMI and this milk component. In fact, the present GWAS helped increase the knowledge of the genetic architecture of such a complex trait, which relies on several factors such as blood glucose availability and uptake, blood–milk barrier (i.e., osmotic equilibrium), and the epithelial integrity of alveoli (i.e., udder health).

Lactose Yield

Findings related to GWAS for LY are shown in Table 5 and the quantile-quantile plot is reported in Figure 1. Visually, 2 major significant peaks were detected for LY (Figure 3); indeed, around 74% of significant variants were within BTA6 and BTA14, and the amount of genetic variance explained by the top 5 significant SNP was 8.45%, with the variant within *ADGRB1* accounting for 2.44% (Figure 4). The latter is a regulator of transmembrane signaling receptor activity, promotor of microbicidal activity of macrophages and associated with udder morphology in cows (Marete et al., 2018), stressing the strong biological dependence of LY on udder health and immune response. The same variant was the most significant in a GWAS for mastitis (Wang et al., 2015) and for milk yield and fat and protein percentage in Chinese Holsteins (Wang et al., 2019). Sahana et al. (2013) found the above-mentioned SNP

Table 2. Significant SNP for deregressed EBV of lactose percentage, their position on *Bos taurus* autosomes, and *P*-values

BTA	SNP	Position (Mb)	*P*-value
1	ARS-BFGL-NGS-5124	71.263427	9.293E-05
	Hapmap42521-BTA-35582	72.736590	4.123E-06
	ARS-BFGL-NGS-95240	154.087389	3.260E-05
2	ARS-BFGL-NGS-39978	5.757355	4.132E-08
	Hapmap49624-BTA-47893	6.700805	4.093E-05
3	ARS-BFGL-NGS-64215	15.525599	4.367E-05
7	ARS-BFGL-NGS-110962	1.009369	5.051E-07
12	BTA-123122-no-rs	69.319934	1.920E-07
	Hapmap50646-BTA-29027	70.140996	2.090E-05
	ARS-BFGL-NGS-57541	77.315938	9.680E-05
16	ARS-BFGL-NGS-74373	51.811400	9.342E-05
	BTA-26576-no-rs	67.703949	2.502E-05
18	ARS-BFGL-NGS-119782	34.126956	2.138E-06
19	ARS-BFGL-NGS-19774	44.547216	5.576E-05
	Hapmap25852-BTA-148919	61.016756	1.027E-05
	ARS-BFGL-NGS-55564	61.441042	1.371E-05
20	BTB-01648514	58.240835	2.366E-06
	BTB-01648552	58.264762	9.257E-05
28	BTB-00874839	6.547497	6.825E-05
	BTB-00874898	6.575192	4.084E-07
	ARS-BFGL-NGS-40170	6.888276	1.618E-05
29	Hapmap32898-BTA-66437	9.319793	4.730E-06

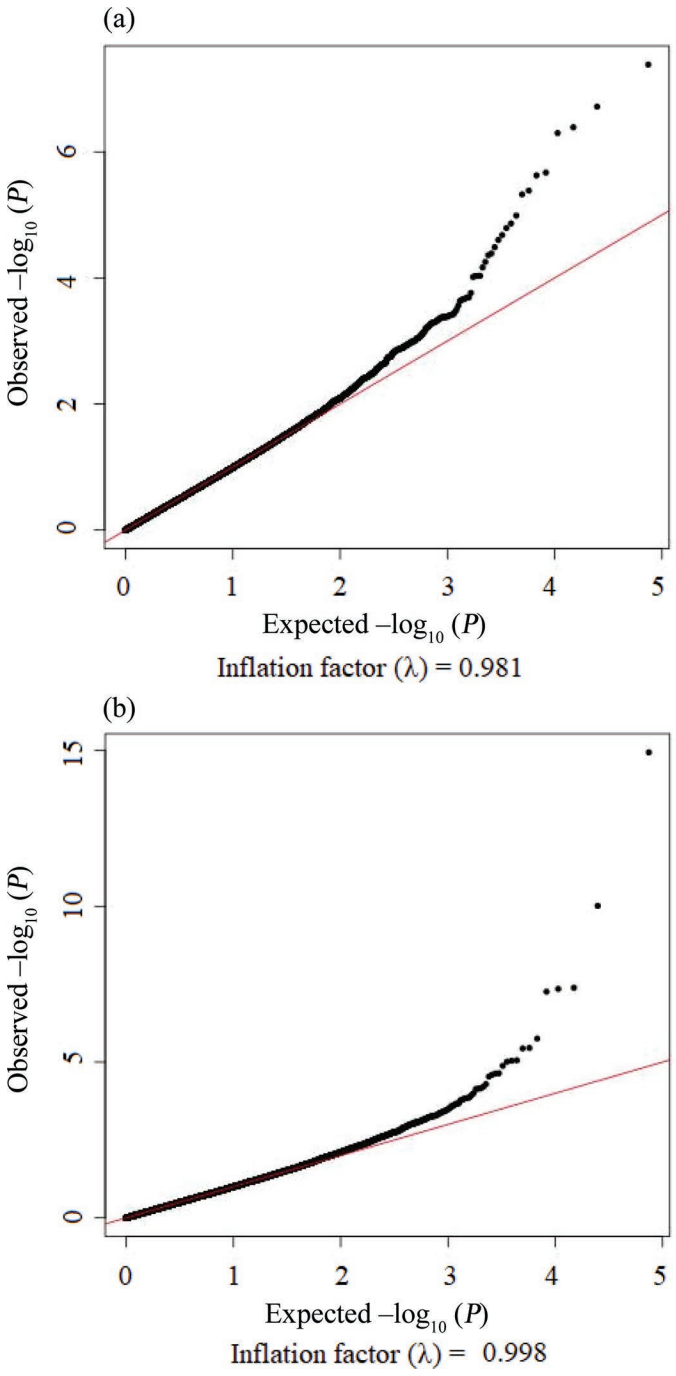

Figure 1. Quantile–quantile plot of the expected *P*-values under null hypothesis versus the observed *P*-values distribution for (a) lactose percentage, and (b) lactose yield.

to be significant for mastitis in second-parity cows. Interestingly, the *DGAT1* gene was found to influence LY in the GWAS of Lopdell et al. (2017), based on the UMD3.1.1 assembly. Because the current GWAS was based on the ARS-UCD1.2 genome assembly, *DGAT1* was not identified in this study; however, the presence of such a significant signal confirmed previous findings on the presence of significant QTL for LY around 1.77 Mb of BTA14 (tag variant rs134364612; Lopdell et al., 2017).

A list of all detected genes is provided in Table 3, with their main functions listed in Table 6. Few regions on BTA6 and BTA14 are known to affect SCS and mastitis (Ogorevc et al., 2009; Wang et al., 2015). Within BTA6, several signals covered a region from 84.57 to 88.82 Mb, coincident to the window already reported to affect mastitis or SCS in US Holsteins (Cole et al., 2011), Nordic Holsteins (Sahana et al., 2013), Danish Holsteins (Wu et al., 2015), and German Holsteins (Abdel-Shafy et al., 2018). Furthermore, a variant in position 88.82 Mb of BTA6 was very close to *CXCL8* (88.81 Mb), a gene related to mastitis, IMI, immune and inflammatory responses, and neutrophil activation (Youngerman et al., 2004; NCBI, 2018). Moreover, this significant region (87.11 to 88.82 Mb, BTA6) included *NPFFR2* (87.25 to 87.33 Mb), which is involved in macrophage activity stimulation, and it is related to udder health. In fact, several authors (Sahana et al., 2013; Wu et al., 2015; Cai et al., 2018) reported a relationship between mastitis and *NPFFR2* in bovines. Pausch et al. (2016) found this gene to affect both the morphology of mammary gland and mastitis resistance in FV cows. Within the above-mentioned window of BTA6, 3 other important genes were detected: *MGC152010*, associated with blood NEFA and metabolic status of cow (Ha et al., 2015), and *ANKRD17* and *LOC781441*, which are related to neutrophil count and activity and to glucuronosyltransferase activity, respectively. The gene *ANKRD17* was less than 0.1 Mb from *COX18*, located in an area significant for SCS in dairy cows (Chen et al., 2015). The significant gene *SLC4A4* (also known as *NBCe1*) is a sodium-cotransporter solute carrier, patented as a genetic marker for mastitis resistance (Yamaguchi and Ishikawa, 2008; Fang et al., 2017, 2018) and was flanking the region on BTA6 detected in this GWAS. Our results are supported by those of Fang et al. (2017), who reported a significant region for mastitis resistance at 88.84 and 88.72 Mb (BTA6) for Holstein and Nordic Red cows, respectively. The variant Hapmap25708-BTC-043671 (87.11 Mb) was less than 1 Mb from *DCK*, which is related to udder health (Wu et al., 2015; Cai et al., 2018), milk protein (Strucken et al., 2012), and milk casein (Dadousis et al., 2017).

On BTA14, a significant SNP (4.34 Mb) was close to *FAM135B*, involved in cellular lipid metabolic processes, and *COL22A1*, a gene that was significant for milk and protein yield and fat percentage in the study of Jiang et al. (2010) in Chinese Holstein cows. Furthermore, on the same chromosome, the sugar transport regulating gene *SLC45A4* and a regulator of ketone body metabo-

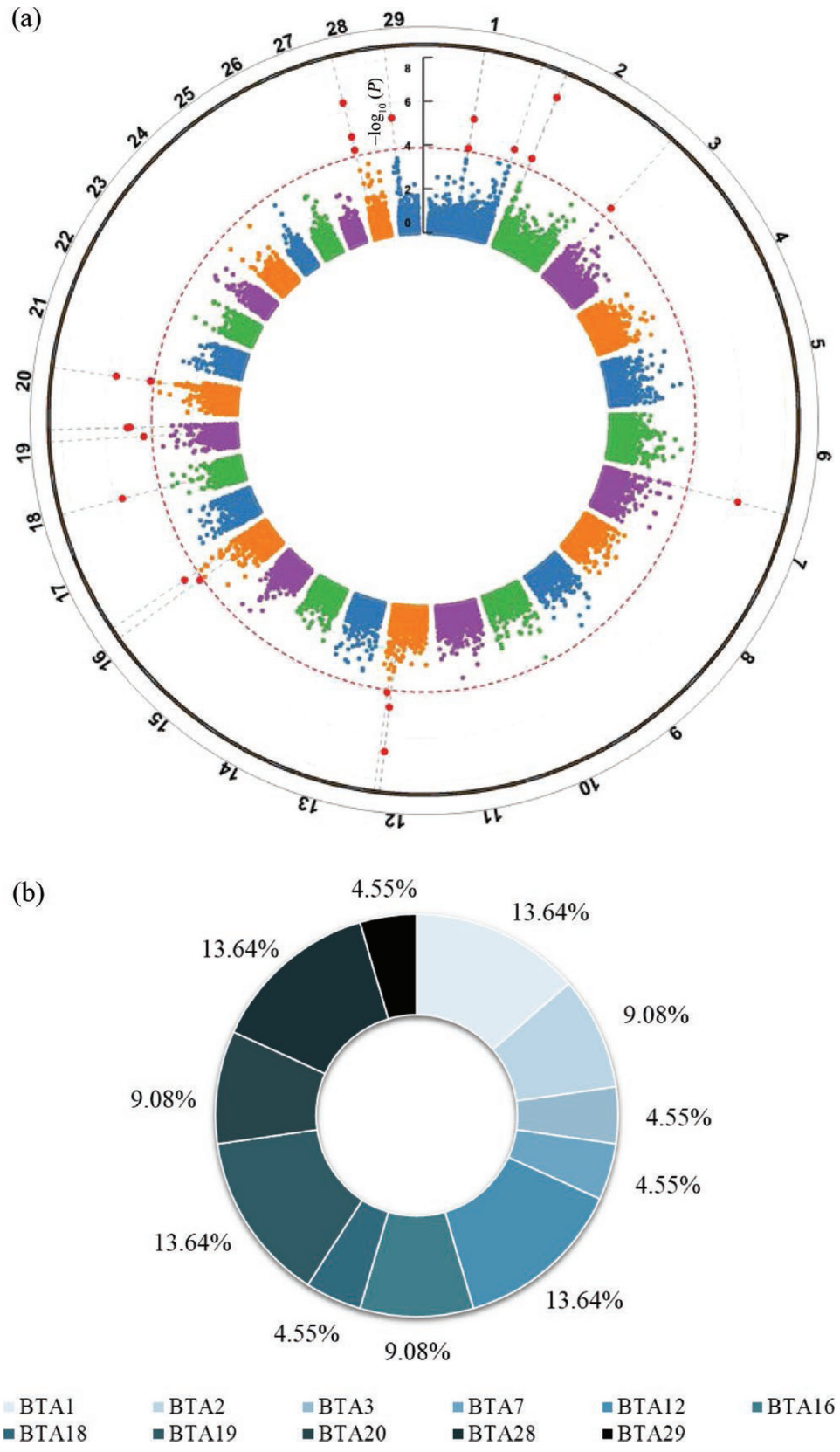

Figure 2. (a) Manhattan plot for lactose percentage, and (b) distribution of significant variants across BTA. The gray solid and red dashed lines in the Manhattan plots indicate BTA and false discovery rate threshold ($P < 0.00013$), respectively.

Table 3. Gene harboring the SNP ($Gene_H$) and nearby genes (left and right) for each significant SNP for lactose percentage (LP) and lactose yield (LY)

Trait	BTA	SNP	$Gene_H$	Nearby genes (±0.1 Mb from SNP)
LP	1	ARS-BFGL-NGS-5124	*PAK2*	*NRROS*, *CEP19*, *LOC112447342*, *PIGX*, *SENP5*, *LOC112447342*, *LOC104970886*
LP	1	ARS-BFGL-NGS-95240	*PLCL2*	*TBC1D5*
LP	1	Hapmap42521-BTA-35582	—	*LOC104970891*
LP	2	ARS-BFGL-NGS-39978	—	*NEMP2*, *NAB1*, *MFSD6*, *LOC104971101*
LP	2	Hapmap49624-BTA-47893	—	*ANKAR*, *OSGELP1*, *ASNSD1*, *SLC40A1*
LP	3	ARS-BFGL-NGS-64215	—	*EFNA1*, *EFNA3*, *EFNA4*, *ADAM15*, *DCST1*, *DCST2*, *ZBTB7B*, *LENEP*, *FLAD1*, *CKS1B*, *SHC1*, *LOC107132270*, *MIR92B*, *MUC1*, *TRIM46*, *KRTCAP2*, *DPM3*, *SLC50A1*
LY	6	ARS-BFGL-NGS-17376	—	*LOC112447100*, *LOC112447101*, *LOC107132573*, *CXCL8*, *LOC534181*, *LOC112447102*, *CXCL5*, *PPBP*
LY	6	ARS-BFGL-NGS-27958	*MGC152010*	*LOC781441*, *LOC540544*, *LOC781478*, *LOC530553*, *LOC530356*
LY	6	BTA-111700-no-rs	*LOC781441*	*LOC104968908*, *LOC100296421*, *LOC781649*, *LOC540544*, *LOC781478*, *MGC152010*
LY	6	BTA-77173-no-rs	*ANKRD17*	*COX18*
LY	6	Hapmap25708-BTC-043671	—	—
LY	6	Hapmap42671-BTA-77163	—	*LOC112447164*, *LOC112447182*
LY	6	Hapmap54336-rs29010419	—	*COX18*, *LOC112447164*, *ANKRD17*
LY	6	Hapmap57014-rs29019575	—	*ADAMTS3*, *TRNAC-GCA*
LP	7	ARS-BFGL-NGS-110962	*RASGEF1C*	*MAPK9*, *GFPT2*, *LOC107132588*, *LOC100848388*
LP	12	ARS-BFGL-NGS-57541	*LOC112449084*	*TMTC4*, *GGACT*, *MIR2892*
LP	12	BTA-123122-no-rs	—	—
LP	12	Hapmap50646-BTA-29027	*LOC784305*	—
LY	14	ARS-BFGL-NGS-112858	—	*KHDRBS3*
LY	14	ARS-BFGL-NGS-3122	—	*SLC45A4*, *DENND3*, *LOC112449594*, *LOC112449595*
LY	14	ARS-BFGL-NGS-34135	*LYNX1*	*LOC112441461*, *LOC112449566*, *LOC112449590*, *LOC100848939*, *LOC104973965*, *LY6D*, *LOC112449567*, *LYPD2*, *SLURP1*, *THEM6*, *PSCA*, *JRK*, *ARC*, *LOC101905222*
LY	14	ARS-BFGL-NGS-4939	*ADGRB1*	*PSCA*, *JRK*, *ARC*, *LOC101905222*
LY	14	ARS-BFGL-NGS-56327	—	*COL22A1*, *FAM135B*, *LOC112449648*
LY	14	BTA-35941-no-rs	—	*LOC101905853*, *LOC101901918*, *TRNAC-GCA*
LY	14	Hapmap23302-BTC-052123	—	—
LY	14	Hapmap23454-BTC-046932	—	—
LY	14	Hapmap26283-BTC-048098	—	—
LY	16	ARS-BFGL-NGS-15423	—	*LOC112441794*
LY	16	ARS-BFGL-NGS-2382	—	*DSTYK*, *CNTN2*, *TMEM81*, *RBBP5*, *TMCC2*, *LOC112441810*
LP	16	ARS-BFGL-NGS-74373	*LOC101904639*	*SPEN*, *LOC507787*, *LOC789035*, *FBLIM1*, *TMEM82*, *SLC25A34*, *LOC112441770*, *PLEKHM2*, *LOC515551*, *LOC112441934*
LP	16	BTA-26576-no-rs	—	*PTGS2*, *LOC107133257*, *LOC112441859*
LY	17	ARS-BFGL-NGS-14166	*ARHGAP10*	—
LY	17	BTB-00689316	—	*RSPH14*, *GNAZ*, *LOC531152*, *VPREB1*, *TOP3B*, *PPM1F*, *MAPK1*, *LOC112442124*
LY	18	ARS-BFGL-NGS-114779	*ZNF423*	*TRNAG-CCC*, *LOC112442280*
LP	18	ARS-BFGL-NGS-119782	*CDH5*	*BEAN1*
LP	19	ARS-BFGL-NGS-19774	*GJC1*	*DBF4B*, *HIGD1B*, *EFTUD2*, *MIR2343*, *CCDC103*, *FAM187A*, *GFAP*, *KIF18B*, *LOC104975097*
LP	19	ARS-BFGL-NGS-55564	*ABCA6*	*ABCA5*, *ABCA9*, *ABCA10*
LP	19	Hapmap25852-BTA-148919	*LOC112442737*	—
LP	20	BTB-01648514	—	*ANKH*, *LOC107131578*, *LOC112443072*
LP	20	BTB-01648552	—	*ANKH*, *LOC107131578*, *LOC112443072*
LY	21	Hapmap58004-rs29023371	—	*LOC112443345*
LP	28	ARS-BFGL-NGS-40170	*SLC35F3*	—
LP	28	BTB-00874839	—	*KCNK1*, *TRNAC-ACA*
LP	28	BTB-00874898	—	*KCNK1*, *TRNAC-ACA*
LP	29	Hapmap32898-BTA-66437	—	*ACTN2*, *HEATR1*, *LOC112444786*, *LOC101908221*

lism, *DENND3*, were both close to the variant ARS-BFGL-NGS-3122 (Sigdel et al., 2017). According to Ogorevc et al. (2009), several mastitis and SCS-related regions are spread across the bovine genome and many are concentrated on BTA14; in fact, *DENND3* was also significant in a GWAS of SCS in dairy cows (Chen et al., 2015). One significant variant was within *LYNX1* and near *LY6D*, *THEM6*, *PSCA*, *SLURP*, and *LYPD2*;

Table 4. Location and main functions of detected genes in genome-wide association study of lactose percentage (sources: Gene Cards, 2018, https://www.genecards.org/; NCBI, 2018)

BTA	Gene	Window (Mb)	Function
1	*PAK2*	71.257814–71.350400	Signal transduction; ATP binding
1	*PLCL2*	153.882096–154.095595	Leukocyte B proliferation; intracellular signal transduction; immune response regulation
7	*RASGEF1C*	1.004381–1.109795	Regulation of membrane-associated molecular activity; intracellular signaling pathways; cell differentiation and proliferation; cytoskeletal organization; vesicle trafficking; nuclear transport
12	*LOC112449084*	77.289796–77.346416	—
12	*LOC784305*	69.969856–70.238238	Multidrug resistance-associated protein
16	*LOC101904639*	51.805664–51.815138	—
18	*CDH5*	34.122721–34.161590	Calcium-dependent cell adhesion protein; regulation of cell polarity
19	*ABCA6*	61.413594–61.483297	Membrane-associated protein; regulation of extra-/intracellular transport of various molecules; macrophage lipid homeostasis; antimicrobial activity
19	*GJC1*	44.522640–44.555661	Junction protein; cell communication
19	*LOC112442737*	60.983169–61.075519	—
28	*SLC35F3*	6.720734–7.155385	Solute carrier; thiamine transport

all of these genes overlapped with those reported by Tiezzi et al. (2015) for clinical mastitis in dairy cows and are involved in regulation of neutrophil activity. These genes are known as globally as the lymphocyte-antigen-6 complex. This finding supported the idea that LY is strongly dependent on mammary epithelial cell number and functionality. It is worth noting that LY and mastitis are positively associated in FV cows (genetic correlation of 0.52; Costa et al., 2019a), suggesting that high-producing cows are more susceptible to IMI and udder health problems than low-producing cows. One variant was found within *ARHGAP10*, a gene related to mastitis resistance in cows (Kurz et al., 2019), whereas the transcription factor *ZNF423* was detected in BTA18. No coding genes were present in other significant regions of BTA16 and BTA17.

To adjust for the effects of the 2 highest significant signals for LY (P-value <1.00E-10); that is, ARS-BFGL-NGS-4939 (BTA14) and Hapmap25708-BTC-043671 (BTA6), and to check for the presence of effective causal variants, an additional GWAS was performed by fixing the 2 variants in the original model using GEMMA software (Zhou and Stephens, 2012). The significance of variants was checked following the

Table 5. Significant SNP[1] for deregressed EBV of lactose yield, their position on *Bos taurus* autosomes, and P-value

BTA	SNP	Position (Mb)	P-value
6	BTA-111700-no-rs	84.575241	1.763E-06
	ARS-BFGL-NGS-27958	84.689991	3.652E-06
	Hapmap25708-BTC-043671	87.113639	9.683E-11
	Hapmap57014-rs29019575	87.801255	2.923E-05
	Hapmap42671-BTA-77163	88.006286	5.119E-05
	Hapmap54336-rs29010419	88.132026	9.712E-06
	BTA-77173-no-rs	88.242415	1.319E-05
	ARS-BFGL-NGS-17376	88.822266	9.083E-06
14	ARS-BFGL-NGS-34135	1.675278	4.123E-08
	ARS-BFGL-NGS-4939	1.801116	1.152E-15
	BTA-35941-no-rs	2.276443	4.492E-08
	ARS-BFGL-NGS-3122	2.721633	6.219E-05
	ARS-BFGL-NGS-56327	4.336714	3.533E-06
	Hapmap23302-BTC-052123	4.848750	5.512E-08
	Hapmap23454-BTC-046932	5.831267	8.790E-06
	ARS-BFGL-NGS-112858	6.589274	2.578E-05
	Hapmap26283-BTC-048098	7.371252	7.145E-05
16	*ARS-BFGL-NGS-2382*	2.933483	6.967E-05
	ARS-BFGL-NGS-15423	74.158269	2.290E-05
17	ARS-BFGL-NGS-14166	10.283664	2.350E-05
	BTB-00689316	71.925055	7.369E-05
18	ARS-BFGL-NGS-114779	18.192027	9.987E-05
21	*Hapmap58004-rs29023371*	62.069305	1.146E-04

[1]SNP in italics were significant in the additional genome-wide association study adjusted for the 2 most significant (P-value <1.00E-10) variants: Hapmap25708-BTC-043671 and ARS-BFGL-NGS-4939.

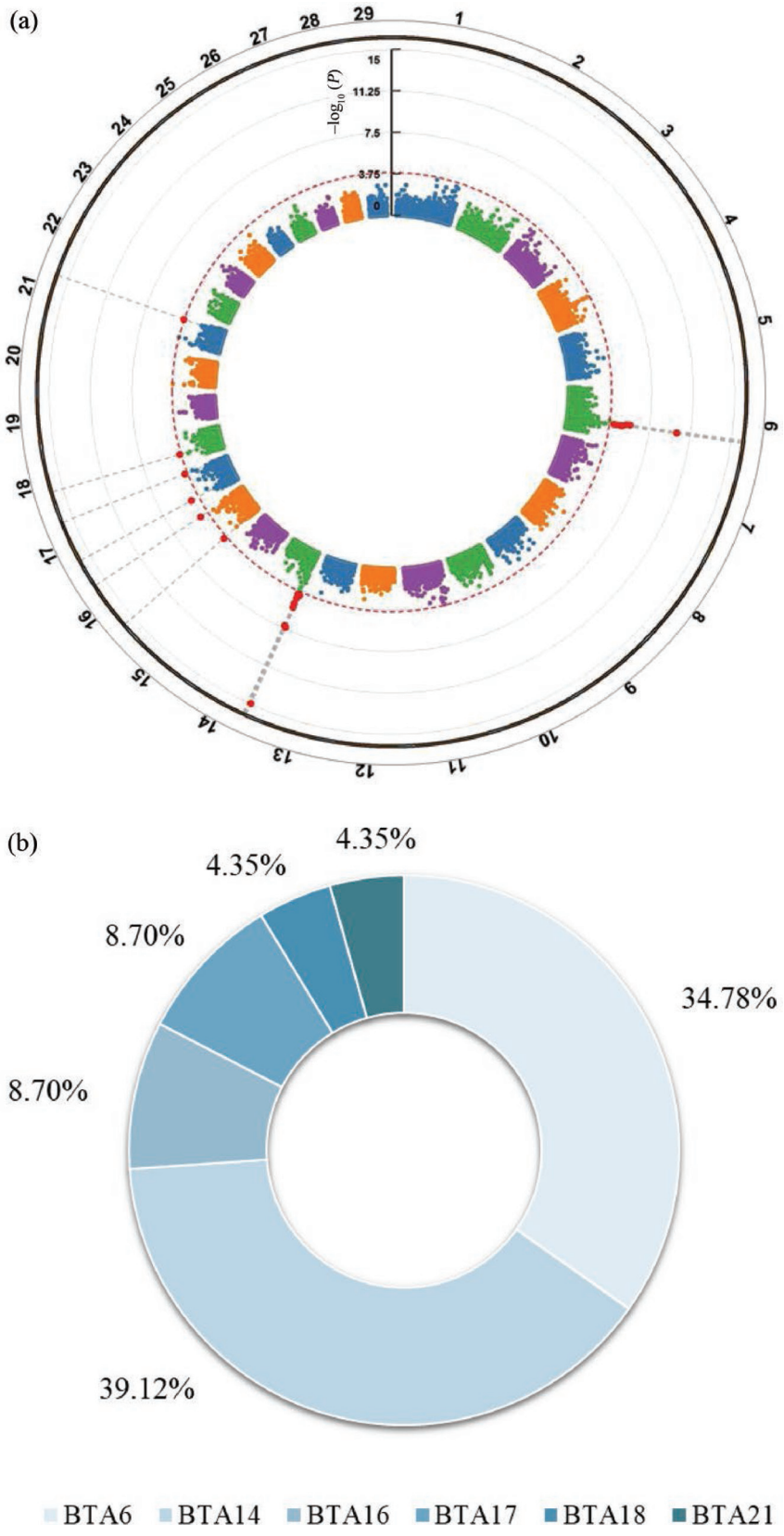

Figure 3. (a) Manhattan plot for lactose yield, and (b) distribution of significant variants across BTA. The gray solid and red dashed lines in the Manhattan plots indicate BTA and false discovery rate threshold ($P < 0.00013$), respectively.

Table 6. Location and main functions of detected genes in genome-wide association study of lactose yield (sources: Gene Cards, 2018, https://www.genecards.org/; NCBI, 2018)

BTA	Gene	Window (Mb)	Functions
6	*LOC781441*	84.563427–84.592360	Glucuronosyltransferase activity
6	*MGC152010*	84.674163–84.712898	Glucuronosyltransferase activity
6	*ANKRD17*	88.187894–88.355145	Ankyrin repeat-containing proteins; related to neutrophils count and activity
14	*LYNX1*	1.669740–1.675364	Acetylcholine receptor binding and regulation; ion channel inhibitor activity
14	*ADGRB1*	1.797345–1.874927	Adhesion G protein-coupled receptor B1, transmembrane signaling receptor activity
17	*ARHGAP10*	10.019895–10.404767	Activation of GTPases RhoA and Cdc42; PTKB2 (Rho GTPase activating protein 10) regulation of cytoskeletal organization via Rho family GTPases
18	*ZNF423*	17.963192–18.309039	Transcription factor, differentiation of white and brown adipocytes; regulation of transforming growth factor (TGF)-β receptor signaling

previously described criteria. Although the number of significant SNP decreased (Table 5), the significant regions detected mirrored those of previous GWAS, with the only exception of a new variant in a noncoding region of BTA20 (4.40 Mb), Hapmap54098-rs29010434. The smaller number of significant polymorphisms was expected, because the 2 strongest signals are thought to be in linkage disequilibrium with other neutral SNP and are also thought to affect the expression of other genes. However, these findings indicated the presence of multiple causal variants segregating in both BTA6 and BTA14 for LY.

CONCLUSIONS

In the present study, we report significant regions of the bovine genome affecting milk lactose in Fleckvieh cattle, the major dairy breed in Austria. The ultimate goal of this study was to detect causal variants and overlapping regions with mastitis and SCS, because lactose percentage and its derived traits have the potential to be used as indicator traits in breeding programs to improve cow udder health. Signals of lactose percentage are scattered across several BTA and connected to some previously identified regions related to intra- or extracellular transport mechanisms, mastitis, and immune response. Conversely, the significant SNP for lactose yield are mainly concentrated on BTA6 and BTA14, always within or close to genes with functions related to transport mechanisms, mastitis, IMI, and inflammatory response. These findings highlight that lactose percentage is affected by several regions of the genome, whereas lactose yield is influenced by fewer regions with larger effects. Lactose yield and percentage do not share common regions, likely because of the different nature of these traits; in fact, the amount of lactose produced is only moderately genetically correlated with the final percentage in milk. Even though lactose percentage and yield are influenced by different regions of the genome, both traits have strong connections with intra- or extracellular transport mechanisms and immune response of dairy cows. Finally, some regions with unknown functions and not previously detected by other GWAS show high significance in the present study.

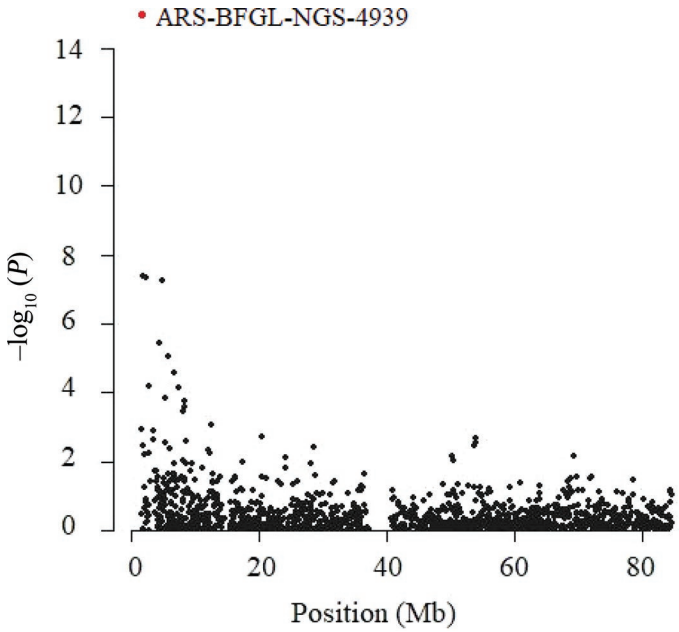

Figure 4. Manhattan plot focused on BTA14 of lactose yield with identification of the SNP (red dot) within *ADGRB1*.

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General conclusions

In this thesis, milk lactose has been investigated in dairy cattle through different approaches. Phenotypic and genetic studies confirmed the essential osmotic role of LY for the determination of milk volume and linked LY to genomic regions involved in molecular transport. Considering the medium to low heritability and the correlation with MY close to unity, LY does not add new information and thus would be scarcely useful for selection purposes. As regards LP, the estimated heritability was medium to high, according to the statistical approach adopted. Moreover, the genetic correlation with udder health (SCS and mastitis) was confirmed. Furthermore, the genome-wide association study showed LP to be affected by genes and regions with functions related to inflammatory response and cellular transport mechanisms; it is worth highlighting that part of the significant signals of LP and LY were within or nearby (< 0.1 Mb) regions affecting mastitis or SCS in bovine.

Results of the present thesis support that low LP (or sudden changes of LP) are alert for inflammation of the mammary gland and impaired epithelial integrity and thus of potentially mastitic cow. According to selection index theory, indicators should: i) show exploitable genetic variation, ii) be more heritable than the breeding objective, and iii) genetically correlate with the target trait (breeding objective). At this point, one could argue that both phenotypic and genetic variability of LP are too scarce to propose LP as effective and powerful indicator of udder health in cattle. Therefore, currently it would be premature to give solid certainties and propose LP as potential novel indicator to improve udder health and mastitis resistance in dairy cows; however, repeated observations (longitudinal data) of LP within the same cow could be informative and produce novel derived phenotypes. The latter could show greater variability at population level and may be more strongly related to udder health or mastitis than LP itself. In this view, the research on lactose is still open to validate its genetic potential and deepen the biological relation with udder health.

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2017

Costa A., De Marchi M., Cassandro M., & Penasa M. 2017. Phenotypic and genetic aspects of milk freezing point in primiparous Holstein Friesian cows, *Agriculturae Conspectus Scientificus*, 82:175-178.

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Author at a glance

Angela Costa was born in Vicenza (Italy) in 1992. After scientific studies, she enrolled at University of Padova (Italy) in 2011, driven by the passion for animals. With a Bachelor in 2014 (110/110 *cum laude*) and a Master degree in 2016 (110/110 *cum laude*) in Animal Science in the pocket, she started her PhD in Animal and Food Science (Department of Agronomy, Food, Natural resources, Animals and Environment - University of Padova) in October 2016 under the supervision of Prof. Mauro Penasa. The PhD project was focused on genetic and genomic aspects of lactose in bovine milk. In 2017 she was awarded a scholarship by "Fondazione Ing. Aldo Gini" of the University of Padova to spend a 6-month study and research period (February to August 2018) at University of Natural Resources and Life Sciences (BOKU, Vienna, Austria), under the supervision of Prof. Johann Sölkner, Prof. Gábor Mészáros, and Dr. Birgit Fuerst-Waltl. In the same year, she was visiting researcher at Wageningen University and Research (WUR, Wageningen, The Netherlands), supervised by Prof. Henk Bovenhuis for a 3-month research period. She is going to do post-doctoral research in the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova. Her research activity is documented by several scientific papers published in international peer-reviewed journals and presentations at national and international meetings and congresses. She is member of the Animal Science and Production Association (ASPA), the American Dairy Science Association (ADSA), and the European Federation of Animal Science (EAAP). Among personal interests, travelling, art history, and techno, minimal, and goa-progressive music stand out.