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**LARGE-SCALE PHENOTYPING OF BOVINE COLOSTRUM: IMPLICATIONS FOR
QUALITY MONITORING AND GENETIC IMPROVEMENT**

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LARGE-SCALE PHENOTYPING OF BOVINE COLOSTRUM: IMPLICATIONS FOR QUALITY MONITORING AND GENETIC IMPROVEMENT

Arianna Goi

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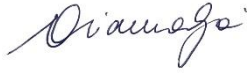
Arianna Goi

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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.

A handwritten signature in dark ink, appearing to read "Di Angelo". The signature is fluid and cursive, with the first name "Di" and last name "Angelo" clearly distinguishable.

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Abstract

Bovine colostrum (BC) is a vital matrix for neonatal calves, providing essential nutrients and bioactive compounds that support growth, early-life development, metabolic activity, and immune system function. The composition of BC is rich in serum proteins, immunoglobulins (Ig)—particularly the isotype G (IgG) which is the main determinant of passive transfer of immunity (PTI). In addition, key components such as lipids, amino acids, and minerals contribute to its nutritional quality. The effectiveness of PTI from the dam to the calf relies on timely ingestion of sufficient volumes of high-quality colostrum, whereas delay, inadequate intake and low IgG content can compromise calf health, increasing disease susceptibility and mortality. However, reference methods for assessing BC quality are laborious, costly, and unsuitable for large-scale or on-farm application, limiting the real-time decision-making and informed management choices, e.g., banking. This motivates the exploration of rapid, non-destructive spectroscopic and analytical approaches to enable accurate and practical evaluation of BC composition, particularly in terms of immunological quality. The overall objectives of the present thesis were: i) to assess BC quality by evaluating both specific quality traits, such as IgG concentration, and broad compositional traits, including gross (protein, fat, lactose) and fine compositional characteristics (essential amino acids and mineral elements); ii) to develop and validate spectroscopic approaches for rapid, non-destructive, cost-effective, and large-scale phenotyping of BC, ranging from laboratory-based mid- and near-infrared devices to portable on-farm tools, and energy-dispersive X-ray fluorescence (ED-XRF); iii) to investigate the effects of non-genetic factors (e.g., parity, calving season, photoperiod at calving, and BC productivity classes) on BC composition, colostrum yield (CY), and quality; iv) to estimate the genetic parameters of CY and quality traits, and evaluate their potential integration into selective breeding programs aimed at improving calves' health and welfare.

Chapter I focuses on evaluating the potential of mid-infrared spectroscopy using a benchtop instrument for predicting IgG, minor Ig isotypes (IgA and IgM), and total Ig concentrations, as well as fat, protein, and lactose content in BC from Italian Holstein cows. In Chapter II, the application of

mid- and near-infrared spectroscopy with benchtop instruments is explored to predict the concentrations of essential amino acids and mineral elements. The phenotypic variability of reference data is analysed to evaluate how the main non-genetic factors, such as parity and calving season, affect these traits. Moreover, correlations between reference data for essential amino acids and mineral elements and Ig fractions are calculated. Chapter III addresses the validation of a portable, on-farm near-infrared spectrometer for real-time BC analysis, covering both IgG concentration and gross and fine composition. Chapter IV investigates the potential of ED-XRF as a novel technique for element quantification, expanding the analytical possibilities beyond infrared methods. Finally, in Chapter V, heritability and genetic correlations are estimated for CY and quality traits, specifically IgG, total Ig, protein, and fat concentrations, in the Holstein population. The same chapter also includes a phenotypic analysis of CY to estimate the influence of parity and photoperiod at calving, and of BC composition traits considering the effects of these factors and BC productivity classes. Using this range of approaches, the study demonstrated practical and cost-effective strategies for evaluating BC quality, providing useful tools for calves' precision feeding, herd management, and potential industrial applications. Moreover, by examining the influence of genetic variability on BC traits, the present thesis offers new insights that could drive genetic selection toward calves' health in Italian Holsteins.

Il colostro bovino (BC) rappresenta una matrice di vitale importanza per i vitelli neonati, fornendo nutrienti essenziali e composti bioattivi che supportano la crescita, lo sviluppo nelle prime fasi di vita, l'attività metabolica e la funzionalità del sistema immunitario. La composizione del BC è particolarmente ricca di proteine sieriche e immunoglobuline (Ig)—in particolare dell'isotipo G (IgG), che rappresenta il principale determinante del trasferimento dell'immunità passiva e della qualità immunologica. Inoltre, elementi fondamentali come lipidi, amminoacidi e minerali contribuiscono congiuntamente a definire la qualità nutrizionale.

L'efficacia del trasferimento dell'immunità passiva dalla madre al vitello dipende dall'ingestione tempestiva di volumi adeguati di colostro di elevata qualità; al contrario, ritardi nella somministrazione, un'assunzione insufficiente o un basso contenuto di IgG possono compromettere la salute del vitello, aumentando la suscettibilità alle malattie e la mortalità. Tuttavia, i metodi di riferimento per la valutazione della qualità del BC risultano laboriosi, costosi e poco adatti all'applicazione su larga scala o direttamente in allevamento, limitando le decisioni gestionali in tempo reale, quali, ad esempio, la gestione delle banche di colostro. Ciò ha motivato l'esplorazione di approcci spettroscopici e analitici rapidi e non distruttivi, finalizzati a una valutazione accurata e pratica della composizione del BC, con particolare attenzione alla qualità immunologica.

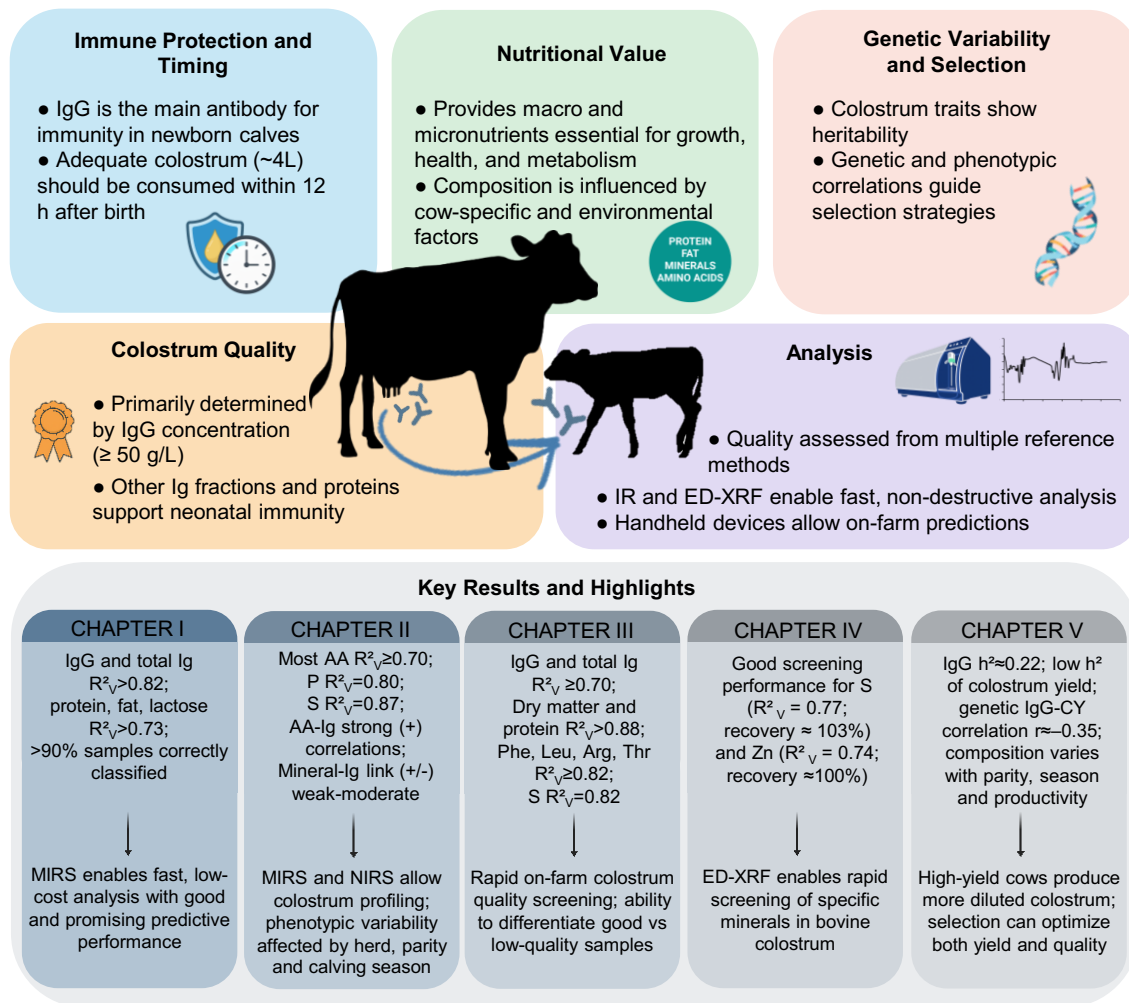
Gli obiettivi generali della presente tesi sono stati: i) valutare la qualità del BC considerando caratteri specifici di qualità, quali la concentrazione di IgG, sia caratteri compositivi di più ampio spettro, comprendenti la composizione chimica di base (proteine, grasso e lattosio) e quella in micronutrienti (amminoacidi essenziali ed elementi minerali); ii) sviluppare e validare approcci spettroscopici rapidi, non distruttivi, economicamente sostenibili e applicabili su larga scala per la fenotipizzazione del colostro, utilizzando strumenti da laboratorio nel medio e vicino infrarosso, dispositivi portatili per uso aziendale e spettrometria a fluorescenza a raggi X a dispersione di energia (ED-XRF); iii) indagare gli effetti dei principali fattori non genetici (ad esempio numero di parto, stagione e fotoperiodo al parto e classi di produttività del colostro) sulla composizione, sulla resa e sulla qualità

del BC; iv) stimare i parametri genetici della resa e dei caratteri qualitativi del BC e valutarne il potenziale impiego in programmi di selezione genetica orientati al miglioramento della salute e del benessere dei vitelli.

Il Capitolo I valuta il potenziale della spettroscopia nel medio infrarosso, eseguita mediante uno strumento da banco, per la predizione delle concentrazioni di IgG, IgA, IgM e immunoglobuline totali, nonché del contenuto di grasso, proteine e lattosio nel colostro di vacche Frisone Italiane. Nel Capitolo II, viene esplorata l'applicazione della spettroscopia nel medio e vicino infrarosso, condotta mediante strumenti da banco, per la predizione delle concentrazioni di amminoacidi essenziali ed elementi minerali. La variabilità fenotipica dei dati di riferimento viene analizzata per valutare l'effetto dei principali fattori non genetici, quali l'ordine di parto e la stagione di parto, su tali caratteri. Inoltre, vengono calcolate le correlazioni tra i dati di riferimento relativi agli amminoacidi essenziali ed elementi minerali e le frazioni immunoglobuliniche. Il Capitolo III riguarda la validazione di uno spettrometro portatile nel vicino infrarosso per l'analisi in tempo reale del colostro in azienda, includendo sia la concentrazione di IgG sia la composizione dei principali costituenti e la composizione in micronutrienti. Il Capitolo IV indaga il potenziale dell'ED-XRF come tecnica innovativa per la quantificazione degli elementi, ampliando le possibilità analitiche oltre i metodi basati sull'infrarosso. Infine, nel Capitolo V, sono stimate l'ereditabilità e le correlazioni genetiche della resa di colostro e dei principali caratteri qualitativi, nello specifico le concentrazioni di IgG, immunoglobuline totali, proteine e grasso, nella popolazione Holstein. Lo stesso capitolo include inoltre un'analisi fenotipica della resa del colostro per stimare l'influenza dell'ordine di parto e del fotoperiodo al parto, nonché un'analisi dei caratteri compositivi del BC considerando gli effetti di tali fattori e delle classi di produttività del colostro.

Attraverso l'impiego di questo insieme di approcci, lo studio ha dimostrato strategie pratiche ed economicamente vantaggiose per la valutazione del BC, fornendo strumenti utili per l'alimentazione di precisione dei vitelli, la gestione della mandria e potenziali applicazioni industriali. Inoltre,

analizzando l'influenza della variabilità genetica sui caratteri del colostro, la presente tesi fornisce nuove indicazioni che potrebbero supportare strategie di selezione genetica orientate al miglioramento della salute dei vitelli nella popolazione Frisona Italiana.



Understanding bovine colostrum: definition, composition, and functional attributes

Colostrum, the initial milk secreted by mammals from the mammary glands after parturition, is a specialized secretion which makes an essential contribution to neonatal health, particularly stimulating the maturation of the infant immune system (Uruakpa et al., 2002). Bovine colostrum (BC), specifically, refers to the milk produced by cows during the first few days postpartum. Its precise definition varies in the literature: some sources restrict it to the first 24 hours (Jaster, 2005), others extend the window to 24–36 hours (Borad and Singh, 2018), or up to 3 days (McGrath, 2016). BC is characterized by significantly higher levels of macronutrients (lipids, proteins, and peptides), micronutrients (vitamins and minerals), and a variety of bioactive compounds (hormones, growth factors, cytokines, nucleotides, probiotics, and prebiotics) compared with milk produced later in lactation, and represents a source of energy and essential molecules (Borad and Singh, 2018). Its composition gradually evolves over time, eventually becoming transitional milk and, subsequently, mature milk. Notably, lactose is an exception, as its concentration is lower in BC and increases in later milk (Arslan et al., 2021). The components of BC support the immune system of the calf, but also have the potential to promote the development and functionality of the newborn's gastrointestinal tract (Westhoff et al., 2024). Furthermore, BC influences the metabolic and endocrine systems, as well as the nutritional state of neonatal calves (Arslan et al., 2021).

The role of colostrum in establishing neonatal immunity

Colostrum represents 0.5% of the total annual milk production of a dairy cow and is primarily allocated for feeding neonatal calves (Costa et al., 2023). This necessity arises from the unique anatomy of the bovine cotyledonary synepitheliochorial placenta, which prevents the acquisition of antibodies in utero and limits the transfer of other bioactive factors from the dam to the foetus (Peter, 2013; Lima et al., 2024). Consequently, calves are born without serum immunoglobulins (Ig) and must acquire them through BC to establish immunity. This process, referred to as passive transfer of immunity (PTI), relies on the absorption of Ig into the calf's bloodstream (Lombard et al., 2020).

Among the antibody classes, the most important in BC are immunoglobulin G (IgG), A (IgA), and M (IgM). Immunoglobulins constitute the majority of colostral proteins, accounting for approximately 70-80% of the total. Among them, IgG represents 85-90% of the overall Ig content, making it the primary determinant of BC quality (Godden, 2008; McGrath et al., 2016). IgA and IgM contribute mainly to short-term immune protection due to their relatively short half-life of 3-4 days, whereas IgG, with a half-life of 21-28 days ensures more extended immunity (Robbers et al., 2021).

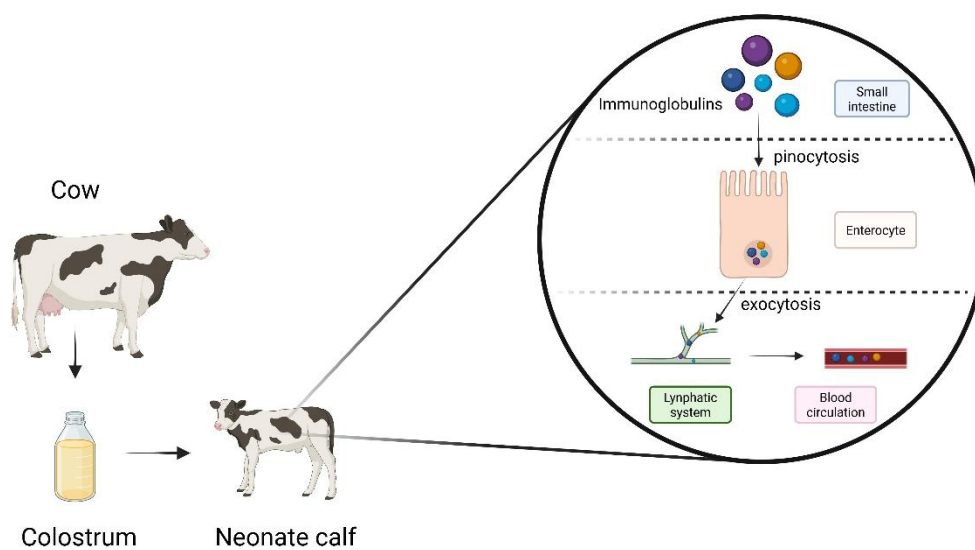
Factors affecting colostrum quality and early-life immune protection

At the farm level, the quality of BC determines the effectiveness of the PTI, which is commonly assessed by measuring IgG or total protein concentrations in the calf's serum approximately 24 hours after BC feeding (Geiger and Lago, 2021). To ensure successful neonatal feeding, minimize the risk of transfer failure, and support immunity, calf serum IgG concentration should ideally exceed 10 mg/mL within 24–48 hours of birth. This target is generally achieved when calves ingest BC containing more than 50 g/L of IgG, whereas lower concentrations indicate BC of poor quality (Godden et al., 2019; Lombard et al., 2020). The efficiency of PTI in calves is influenced by multiple interacting factors, including the timing of ingestion, the IgG concentration and the volume of BC administered, as well as indirect management- and dam-related factors. In particular, the method of BC administration primarily affects the PTI by influencing intake timing and volume, whereas maternal factors contribute to variability in both CY and Ig concentration (Weaver et al., 2000).

To maximize IgG absorption, BC should be administered as early as possible after birth. Ideally, calves should receive their first BC feeding within 1–2 hours of life, and no later than 6 hours postpartum, as intestinal absorptive capacity declines rapidly after birth (Godden, 2008). Additionally, BC composition changes rapidly within the first 24 hours postpartum, with a progressive decline in Ig concentration (Costa et al., 2023). Under practical farming conditions, BC that meets the IgG quality threshold of 50 g/L is generally considered adequate when administered in a standard volume of approximately 4 L within the first 12 hours of life (McGrath et al., 2016).

The biological basis for the importance of early BC feeding is illustrated in Figure 1. Neonatal enterocytes exhibit a distinctive and transient ability to nonselectively absorb macromolecular proteins, including Ig, via pinocytosis. During the first 24-36 hours of life, the Ig absorbed in the small intestine are transported across the enterocytes into the lymphatics through exocytosis and subsequently enter the circulatory system via the thoracic duct (Broughton et al., 1970; Staley et al., 1972).

Figure 1. Schematic representation of immunoglobulin absorption from colostrum in neonatal calf.



The macromolecule absorption process progressively declines and its cessation, termed closure, occurs in calves around 24 hours postpartum, although absorption capacity may be extended to approximately 36 hours if BC feeding is delayed (Weaver et al., 2000). Despite the duration of gut permeability, Ig absorption is most efficient within the first 4 hours after birth, with a sharp decline observed after 12 hours. Consequently, calves fed earlier achieve significantly higher serum IgG concentration compared to those fed later, when BC of similar volume and concentration is provided (Godden, 2008). Therefore, timely ingestion of adequate volumes of high-quality BC shortly after birth is essential to achieve optimal circulating IgG concentrations and ensure effective PTI. Elevated serum IgG levels in calves are strongly associated with reduced morbidity and mortality, making IgG

concentration a reliable predictor of overall calf health. In fact, failure to PTI has been linked to a higher risk of various health issues, including a 2.12-fold increase in mortality, a 1.75-fold rise in pneumonia cases, a 1.51-fold increase in diarrhoea cases, and a 1.91-fold rise in overall morbidity (Raboisson et al., 2016).

Regarding BC feeding, both natural suckling and assisted or active administration are used, with the first method associated with an increased risk to the neonate due to the inability to control volume and quality of the BC ingested. The scientific literature does not present a unanimous consensus on which methods are more effective in ensuring higher serum IgG concentrations and in reducing the risk of failure of PTI, defined as serum IgG < 10 mg/mL (Stott et al., 1979; Besser et al., 1991; Robbers et al., 2021; Abdelfattah et al. 2025). A key limitation of natural suckling is the potential delay in the initiation of feeding, as the time required for calves to voluntarily begin suckling may exceed 6 hours post-birth (Laestander, 2016). This delay can thus be mitigated—and transfer of IgG improved—through assisted suckling techniques, which involve guiding the calves to the udder (Robbers et al., 2021). However, precise monitoring of volume and Ig concentration is allowed when active administration strategies (e.g., bottle or oesophageal tube) are applied, enabling a greater control (Robbers et al., 2021).

Substantial variability in CY at first milking has been reported among individual dams, across herds, and across different genetic background (Arslan et al., 2021; Soufleri et al., 2021; Westhoff et al., 2024). This variability may limit the total amount of IgG delivered to the neonate, highlighting that both BC quantity and quality are critical determinants of successful PTI. Similarly, BC composition is affected by individual factors, with studies reporting that approximately 21–29% of cows produce colostrum of poor quality (IgG < 50 g/L; Bartier et al., 2015; Westhoff et al. 2023).

Moreover, the dam's health status plays a crucial role in determining BC quality. Inflammatory processes in the mammary gland may modify BC composition through multiple mechanisms, including impaired synthesis of key components, increased permeability of the blood–colostrum

barrier, and elevated proteolytic activity (Puppel et al., 2020). Such alterations can lead to decreased levels of Ig, particularly IgG which are allowed to move from colostrum to the bloodstream due to a disruption of the blood-milk barrier. Beyond health, other factors such as parity have been associated with variations in IgG concentration in BC. Evidence suggests an increasing trend in IgG levels with advancing parity, potentially affecting IgG absorption efficiency in calves. Studies indicate greater IgG concentration in BC from multiparous cows compared to primiparous cows; however, findings remain inconsistent regarding the parity order at which a significant increase in IgG concentration occurs (Dunn et al., 2017; Kessler et al., 2020; Costa et al., 2021b; Costa et al., 2023).

Analytical approaches for assessing colostrum quality and IgG concentration

Given the pivotal role of IgG in determining BC quality and ensuring effective PTI, accurate quantification of IgG is essential. Besides supplying essential antibodies, BC provides primary energy, representing the first source of carbohydrates, fats, and proteins for growth and development. In addition, BC also provides essential amino acids and minerals, which together constitute the so-called fine composition. Although present in varying amounts, both amino acids and minerals are crucial determinants of neonatal health, in fact essential amino acids sustain rapid protein synthesis and contribute to immune activity, while minerals are indispensable for structural functions, homeostatic balance, and numerous biochemical pathways (Li et al., 2020; National Academies of Sciences, Engineering, and Medicine, 2021).

Beyond BC importance for calf development, it has gained growing interest in the scientific, veterinary, and pharmaceutical sectors, particularly for its potential applications as a nutraceutical ingredient for both humans and animals (Goi et al., 2026). One factor contributing to this interest is the practice of colostrum banking, considered a key management strategy to reduce calf mortality and disease risk. In herds where colostrum is banked, excess BC that is not immediately needed by newborns can be frozen and stored for later use. This stored BC can then be administered when production is limited, IgG levels are below the recommended threshold, or female calves intended for

future herd replacement are born (Costa et al., 2023). Such management practice, especially on efficiently managed farms where storage capacity is reached, results in surpluses that can be utilized for the production of various BC preparations, including powders, tablets, capsules, and liquids (Bagwe et al., 2015). In fact, according to Costa et al. (2023), since 2000, both the demand and value of BC have risen significantly, and by 2001, the retail market for BC was valued at approximately USD 100 million, offering potentially profitable opportunities for both farmers and manufacturers. Additionally, the growing research relevance of BC is evidenced by the publication of over 1,000 scientific articles on the topic between 2010 and 2020 (Franzoi et al., 2022).

Assessing gross and fine composition at a reasonable cost is therefore pivotal to optimize precision feeding of calves, support veterinary practises on farm, and ensure the safety and standardization of foodstuffs, supplements, and medicaments containing BC and entering the market for human use. For this reason, robust assessment of BC quality should integrate Ig concentration with gross and fine compositional traits, providing the basis for quality monitoring, precision feeding, and large-scale phenotyping approaches.

Gold-standard methods

Several methodologies, differing in cost, analysis duration, repeatability, ease of implementation in routine applications with minimal expertise, and overall accuracy, have been developed to quantify and predict BC quality traits. Quantification of Ig concentration can be achieved through radial immunodiffusion (RID) assay, which is widely recognized as the gold standard for measuring Ig in colostrum due to its high repeatability and reproducibility (Pechova et al., 2019). RID is an antigen–antibody precipitation technique in which antibodies specific to the target immunoglobulin are uniformly incorporated into an agarose gel. A small volume of sample is placed into a well punched into the gel surface, from which the Ig diffuse radially. As diffusion progresses, the target antibodies and antigens interact until they reach equilibrium, forming a visible ring of precipitate. The diameter of this ring is directly proportional to the concentration of the target protein in the sample, thus

allowing accurate quantitative determination (Guidry and Pearson, 1979). Although this method provides highly accurate and consistent results, it is considered labour-intensive and costly—typically exceeding USD 5 per sample or around USD 100 per plate—and requires 24 hours of incubation as well as trained personnel to handle and interpret the results (Costa et al., 2021a). Alternatively, enzyme-linked immunosorbent assay (ELISA) kits are frequently employed for direct Ig quantification (Dunn et al., 2017; Cordero-Solórzano et al., 2022). The ELISA method relies on antigen–antibody specific interaction detected through an enzymatic colour change. The assay is typically carried out on a plate where antibodies are used to capture the antigen of interest. After a sequence of incubation and washing steps, a secondary enzyme-conjugated antibody binds to the antigen–antibody complex and upon addition of an appropriate substrate, the enzyme catalyses a colorimetric reaction that produces a measurable signal, the intensity of which is directly proportional to the Ig concentration in the sample. This approach enables both the detection and quantification of the target Ig fraction in a reproducible and relatively time-efficient manner (Tabatabaei and Ahmed, 2022). RID and ELISA, differ in terms of sensitivity (the ability to detect Ig accurately), specificity (the precision in identifying the target immunoglobulin), and consistency (repeatability and reproducibility), with RID typically providing more reliable outcomes (Gelsinger et al., 2014; Costa et al., 2023). Despite its accuracy, direct quantification of Ig using gold-standard methods is resource-intensive, requiring substantial financial investment, labour, time, and specialized personnel, thus limiting its feasibility for routine use in commercial farming or industrial settings. However, these methodologies remain essential for calibrating and validating indirect measurement techniques (Bartier et al., 2015; Spina et al., 2021; Franzoi et al., 2022).

Reference methods for the gross composition analysis of BC, including crude protein, fat, and lactose, are well established for bovine milk through International Organization for Standardization (ISO) and International Dairy Federation (IDF) official standards and have also been successfully applied to BC samples. For crude protein, the Kjeldahl method remains the reference technique; it is internationally

recognized as the gold-standard procedure and is standardized by both ISO (ISO 8968-1:2014) and IDF (IDF 20-1:2014). It provides accurate and reproducible quantification of total nitrogen, which is then converted into protein content using a fixed conversion factor. For the determination of fat content in bovine milk, the Röse–Gottlieb procedure is the accepted reference method, standardized by ISO (ISO 23318:2022) and IDF (IDF 249:2022). Regarding lactose, the current international reference method is the enzymatic procedure (ISO 22662:2024 and IDF 198:2024), coupled with high-performance liquid chromatography (HPLC). Extending the analysis to fine composition, conventional analytical techniques for determining the mineral content in milk, such as inductively coupled plasma optical-emission spectrometry (ICP-OES; ISO 15151:2018 and IDF 229:2018) and inductively coupled plasma mass spectrometry (ICP-MS; ISO 21424:2018 and IDF 243:2018), are recognized as reference methods. Finally, the gold-standard method for the determination of total amino acids, following protein hydrolysis and analysis by HPLC, is ISO 4214:2022 and IDF 254:2022. Although these methods provide highly accurate and reliable results, they are resource-intensive and slow, often taking more than one day to complete. They require specialized equipment, qualified and trained personnel, and involve destructive sampling and polluting chemicals. As a result, they are not suited for rapid or large-scale applications in farm settings and can delay timely decision making (Agiomavriti et al., 2024; Warakaulle et al., 2024).

Indirect on-farm methods

The quality of BC can be assessed indirectly using portable devices such as colostrometers and refractometers. A colostrometer operates by placing a small BC sample in a cylinder to determine its specific gravity, which serves as a proxy for IgG concentration. Results are immediately interpretable via a color-coded scale that categorizes colostrum quality as high in green ($\text{IgG} \geq 51 \text{ mg/mL}$), moderate in yellow (IgG between 21 and 50 mg/mL), or low in red ($\text{IgG} \leq 20 \text{ mg/mL}$). In contrast, Brix refractometers, available in both analog and digital formats, measure sucrose concentrations in liquids as Brix percentage. When applied to non-sucrose-containing fluids, such as colostrum or milk,

the Brix percentage approximates total solids concentration. IgG levels can be estimated from these readings with values between 18% and 23% Brix conventionally considered indicative of high-quality colostrum (Quigley et al., 2013). This method offers improved accuracy, less fragility, and reduced susceptibility to temperature fluctuations compared to colostrometers (Quigley et al., 2013). These attributes have led to the widespread adoption of refractometers on farms as a standard approach for evaluating BC quality. Nevertheless, both colostrometers and refractometers are rapid, easy to use on-farm, and exhibit moderate correlation with the gold standard RID assay (Bartier et al., 2015; Morrill et al., 2015).

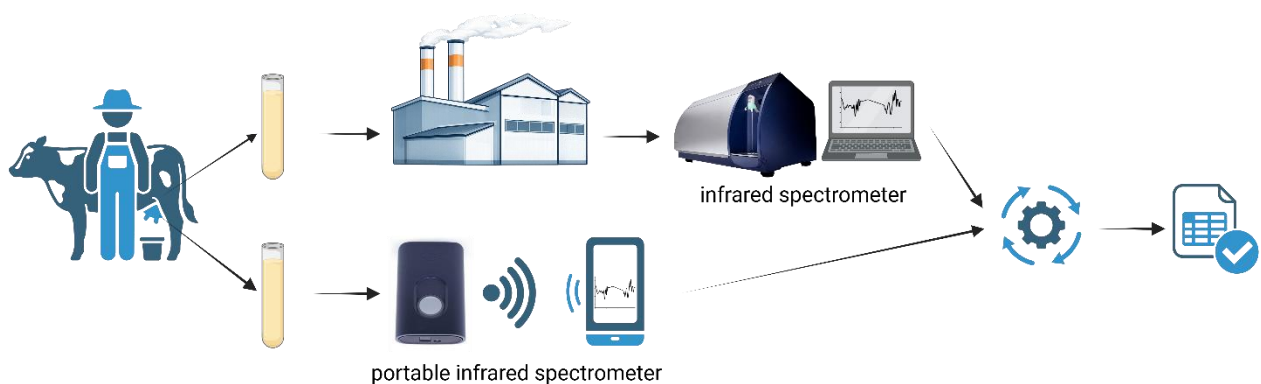
Infrared spectroscopy

Infrared spectroscopy, encompassing mid-infrared (MIR), near-infrared (NIR), and visible–near-infrared (Vis–NIR) techniques, is another analytical approach commonly applied to dairy matrices. MIR spectroscopy is a widely adopted tool among milk recording organizations for the prediction of milk protein, fat, and lactose (McDermott et al., 2016). Unlike colostrometers and refractometers, which provide estimates of a single parameter, benchtop infrared spectrometers enable simultaneous prediction of multiple compositional traits for a single sample, showing strong correlations with reference methods for BC IgG concentration and total protein content (Rivero et al., 2012; Elsohaby et al., 2018; Costa et al., 2022). The principle of this technique relies on the absorption of electromagnetic radiation by organic matter, which induces specific vibrational movements of chemical bonds. Depending on the spectral region under investigation, different molecular features can be explored. NIR spectroscopy, which operates in the spectral range of approximately 12,500–4,000 cm^{-1} (corresponding to 800–2,500 nm), captures overtone and combination bands and enables deeper penetration into the sample; however, the resulting spectra often exhibit broad and overlapping signals that complicate data interpretation (Manley, 2014). Similarly, the Vis–NIR region extends from 25,000 to 4,000 cm^{-1} (400–2,500 nm) covering both visible and NIR radiation. In contrast, MIR spectroscopy explores the range between 4,000 and 400 cm^{-1} (2,500–25,000 nm), where fundamental

vibrational and rotational modes occur, producing clearer and more distinct absorption bands that can provide highly informative molecular fingerprints (Nunes, 2014; Pereira et al., 2019). Infrared spectroscopy requires minimal sample preparation, and the resulting spectra can be used to rapidly and non-destructively estimate multiple traits—Ig, gross and fine composition—in a cost-effective manner. Previous studies have demonstrated reliable prediction of colostrum IgG and major compositional traits using this approach; however, only a limited number of investigations have examined finer compositional components, such as amino acids and minerals, particularly using large datasets.

Alongside traditional benchtop instruments, portable and pocket-sized infrared spectrometers have recently become an important tool to support fast analysis and real-time decision-making for farmers and operators in the dairy sector, due to their ease of use and affordability (Costa et al., 2023). The workflow of laboratory-based and portable infrared analysis is illustrated in Figure 2. This technological innovation in quality and IgG concentration assessment offers new opportunities for on-site BC analysis, paving the way for standardized and large-scale implementation of these tools.

Figure 2. Schematic representation of colostrum analysis workflow: from sample collection to data processing. Comparison between laboratory-based infrared spectrometry and on-site analysis using portable devices.



Energy-dispersive X-ray fluorescence

Energy-dispersive X-ray fluorescence (ED-XRF) has emerged as a promising alternative for the determination of elemental composition in biological matrices. ED-XRF offers several advantages, including the avoidance of hazardous chemicals and the elimination of time-consuming mineralization steps, making it particularly suitable for analysing samples with high organic matter content, including milk and colostrum (Herrerros-Chavez et al., 2019; Smagunova and Pashkova, 2013). The technique is based on the excitation of the sample by an X-ray pulse, which induces the emission of element-specific fluorescent radiation. This radiation can be analysed qualitatively to identify the elements present, while the measurement of its intensity enables the quantitative determination of their concentrations (Brouwer, 2003). ED-XRF thus provides rapid, non-destructive, and cost-effective elemental quantification, which aligns with the increasing demand for practical tools in both research and industrial contexts. Studies have demonstrated the feasibility of ED-XRF for predicting elemental concentrations in various dairy products, suggesting that it may represent a valuable analytical tool for the fine compositional assessment of BC, supporting both scientific investigation and potential industrial applications (Herrerros-Chavez et al., 2019; Visentin et al., 2023a,b; Pozza et al., 2024).

Genetic perspective on colostrum traits

As highlighted in the preceding sections, BC quality and quantity are shaped by a combination of environmental and management factors, including dam health, nutrition, timing of collection, and feeding strategies. In addition to these influences, genetic differences among individual cows also contribute to the observed variation in BC traits; in fact, heritability estimates from recent studies indicate that these traits are partially heritable and therefore a portion of phenotypic variability in BC is influenced by genetics (Soufleri et al., 2019; Costa et al., 2021a). A better understanding of the genetic basis underlying these traits is of growing interest, as both CY and Ig concentration are essential determinants of PTI, calf health, and early survival. From a breeding perspective, identifying

heritable variation in BC traits could support the development of selection strategies aimed at improving both the volume and the immunological value of the matrix, thereby reducing the incidence of failure of PTI and enhancing animal welfare. Furthermore, higher CY and quality could provide additional benefits at the farm level, including a more efficient use of BC for banking purposes, and potential valorisation of surplus BC in nutraceutical and industrial applications. Despite these potential advantages, the genetic determinism of CY and its relationship with BC quality remain largely unexplored, emphasizing the need for further studies to clarify their heritability and genetic correlations.

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The overall objective of the present thesis is to develop and validate spectroscopic and statistical approaches for the rapid, non-destructive, and cost-effective phenotyping of IgG concentration and quality characteristics of BC. The work primarily focuses on improving the assessment of BC composition and variability, supporting management strategies for PTI in newborn calves, and exploring the potential valorisation of BC as a material for industrial and nutraceutical applications. Additionally, the thesis considers the genetic dimension of BC traits as a perspective to inform future selective breeding strategies.

The first objective focuses on evaluating the potential of MIR spectroscopy as an analytical tool for predicting Ig concentration and gross compositional traits, such as protein, fat, and lactose (Chapter I). Chapter II aims to expand the applications of infrared spectroscopy, encompassing both MIR and NIR approaches, to explore additional compositional traits of BC, including essential amino acid and mineral profiles. Another objective involves the evaluation of a portable, on-farm NIR device for real-time BC analysis (Chapter III). The study investigates its feasibility and accuracy, with the aim of enabling rapid and user-friendly assessment directly on farms, supporting management decisions, and facilitating large-scale data collection for phenotyping purposes. An alternative spectroscopic technology, ED-XRF, is also explored in Chapter IV as a novel application for the characterization of the mineral composition of BC. This technique is tested for its capacity to provide fast and non-destructive screening of mineral elements, with the aim of identifying those that can be reliably quantified for routine or field applications. Finally, Chapter V presents and discusses the genetic parameters of key BC traits, namely yield at first milking and IgG concentration. In particular, heritability and genetic correlations were estimated for the Italian Holstein population using BC composition traits predicted with Vis–NIR models, providing insights into the genetic variability of BC traits and their potential relevance for future research on selective breeding.

**Mid-infrared spectroscopy for large-scale phenotyping of bovine colostrum
gross composition and immunoglobulin concentration**

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Mid-infrared spectroscopy for large-scale phenotyping of bovine colostrum gross composition and immunoglobulin concentration

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ABSTRACT

Immunoglobulin G is the fundamental antibody for acquisition of passive transfer of immunity in ruminant newborns. Colostrum, in fact, must be administered as soon as possible after birth to ensure a successful transfer of IgG from the dam to the calf. Assessment of colostrum Ig concentration and gross composition via gold standards is expensive, time consuming, and hardly implementable for large-scale investigations. Therefore, in the present study we evaluated the predictive ability of mid-infrared spectroscopy (MIRS) as an indirect determination method. A total of 714 colostrum samples collected within 6 h from parturition from Italian Holstein cows, 30% primiparous and 70% pluriparous, were scanned using a benchtop spectrometer after dilution in pure water. The prediction models were developed by correlating spectral information with the reference measurements: IgG concentration (93.54 ± 33.87 g/L), total Ig concentrations (102.82 ± 35.04 g/L), and content of protein ($14.71 \pm 3.51\%$), fat ($4.61 \pm 3.04\%$), and lactose (2.36 ± 0.51 mg/100 mg). We found a good to excellent performance in prediction of colostrum IgG concentration and traditional composition traits in cross-validation ($R^2_{CV} \geq 0.92$) and a promising and good predictive ability in external validation with R^2_v equal to 0.84, 0.89, and 0.74 for IgG, protein, and fat, respectively. In the case of IgG and protein content, for example, the coefficient of determination in external validation was greater than 0.84. The other Ig fractions, A and M, presented insufficient prediction accuracy likely due to their extremely low concentration compared with IgG (4.56 and 5.06 g/L vs. 93.54 g/L). The discriminant ability of MIRS-predicted IgG and protein content was outstanding when trying to classify samples according to the quality level (i.e., low vs. high concentration of IgG). In particular, the cut-off that better discriminate low- from high-quality colostrum

was 75.40 g/L in the case of the MIRS-predicted IgG and 13.32% for the MIRS-predicted protein content. Therefore, MIRS is proposed as a rapid and cheap tool for large-scale punctual IgG, protein, and lactose quantification and for the screening of low-quality samples. From a practical perspective, there is the possibility to install colostrum models in the MIRS benchtop machineries already present in laboratories in charge of official milk testing. Colostrum phenotypes collected on an individual basis will be useful to breeders for the definition of specific selection strategies and to farmers for management scopes. Finally, our findings may be relevant for other stakeholders, given the fact that colostrum is an emerging ingredient for the animal and human food and pharmaceutical industry.

Key words: colostrum, dairy cattle, animal health, calf, passive transfer of immunity

INTRODUCTION

In most ruminants, newborns are agammaglobulinemic at birth and immunity is exclusively reached passively through a proper administration of colostrum. Successful transfer of immunity relies on both the quality and the quantity of the administered colostrum (Godden et al., 2019; Costa et al., 2021a).

The most important Ig isotypes are 3 in bovine colostrum, G (IgG), A (IgA), and M (IgM), with IgG being the key antibodies of cow colostrum and representing nearly 80% of total proteins (McGrath et al., 2016). Portable small devices to be used on farm are available at a reasonable cost to indirectly assess colostrum quality (i.e., to approximate IgG concentration). Refractometers, for example, provide the refractive index (Brix, %) of the analyzed medium, which in colostrum is correlated with total solids and protein content (Soufleri et al., 2019). Various studies have discussed the accuracy of refractometers for cheap and indirect determination of the colostrum narrow-sense quality, i.e., of the IgG concentration usually determined via radial immunodiffusion (RID; Quigley et al., 2013; Soufleri et al., 2019). The Pearson correlation coefficient between Brix

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and RID assay reported by Quigley et al. (2013) and Morrill et al. (2015) is 0.75 and 0.79, respectively, and Biemann et al. (2010) calculated a correlation of 0.71 for the optical refractometer and 0.73 for the digital. These values are very close to the ones reported by Elsohaby et al. (2017): 0.71 (optical refractometer) and 0.72 (digital refractometer). Although strong, the correlation is rather far from unity. In fact, the refractive index is not only given by IgG of colostrum, being more correlated to total solids that include other proteins, lactose, and fat (Morin et al., 2001). Refractometers can be therefore be used for quality screening, but do not allow estimation of a punctual and precise IgG concentration. On the contrary, determination of colostrum antibodies can be successfully achieved through infrared spectroscopy. Very satisfactory prediction accuracies have been obtained by Franzoi et al. (2022) using near-infrared spectroscopy on cow colostrum sampled within 6 h from calving. Moreover, Spina et al. (2021) demonstrated that IgG concentration is strongly correlated with total protein content of colostrum predicted using a mid-infrared spectroscopy (MIRS) prediction model developed for protein content of milk. In general, infrared spectroscopy is based on the study of interactions between electromagnetic waves and organic matter, which generates vibrations of the chemical bonds from all constituents of the substrate. Electromagnetic radiation comprises different regions according to the range of wavelengths (O'Donnell et al., 2014). The main difference between near-infrared spectroscopy and MIRS is that the former uses the shorter near-infrared wavelengths (12,500–4,000 cm^{-1}) that enable increased penetration depth (Manley, 2014) and is based on molecular overtone and combination of such vibrations, while the latter works in the range of the electromagnetic spectrum of 4,000 to 400 cm^{-1} and monitors the fundamental vibrational and rotational frequencies (Nunes, 2014). Irrespective of the methodology, the output of the analysis can be visualized in a so-called spectrum, which is a graphical representation of the energy transmitted (or absorbed) as a function of the incident wavelength. While the near-infrared spectrum contains more overlapped bands that make it more difficult to be interpreted (Reid et al., 2005), the mid-infrared spectrum contains more isolated bands and could be potentially more informative (Roychoudhury et al., 2006; De Marchi et al., 2014; Pereira et al., 2019).

To the best of our knowledge, few studies have investigated the ability of MIRS to predict IgG concentration of cow colostrum collected in the field under commercial conditions and none have evaluated the predictability of gross composition and other Ig isotypes. Elsohaby et al. (2017, 2018) developed a calibration model to

relate the spectra (4,000 and 400 cm^{-1}) to the colostrum IgG concentrations obtained via RID using beef and dairy cows' samples ($n = 430$). The Pearson correlation between predicted and measured IgG was 0.92 in the validation set ($n = 143$).

Such a lack is mainly due to the high cost of wet chemical analyses via gold standards and practical sampling difficulties in commercial farms, such as collecting a sufficient quantity of colostrum immediately after birth, correct labeling, and storage. The definition of a strict standardized protocol is reported to be essential to catch variability of quality of the first colostrum secreted by the mammary gland after parturition (Costa et al., 2021b). Accurate MIRS prediction of IgG (in g/L) may boost the adoption of precision feeding strategies in calves to ensure adequate passive transfer of immunity. Major advantages of MIRS are the possibility to simultaneously assess multiple parameters in a single analysis with just one sample, its use for the analysis of milk within the official recording system worldwide, and the constant routine standardization through ring tests. Development of specific prediction equations for bovine colostrum is advisable and can raise the interest of cost-effective on-line monitoring of composition traits and IgG.

Our objective in the present study was thereby to assess the predictive ability of MIRS for colostrum Ig fraction concentrations and gross composition using data referred to individual colostrum samples of Italian Holstein cows.

MATERIALS AND METHODS

Sampling

Ethical approval was not required for the present study as per institutional guidelines/local legislation, since only farmers interacted with the cows. The animals' owners involved in the study signed a written informed consent and are associated with the Veneto Region Breeders Association (ARAV, Vicenza, Italy). The whole experiment has previously been described by Costa et al. (2021b); briefly, 714 individual samples of colostrum were collected in 9 commercial Holstein farms located in northern Italy. The animals were 215 primiparous and 499 pluriparous cows, with average parity order of 2.47 ± 1.42 . The experiment was designed in such a way to cover variability related to calving season and cow parity (Costa et al., 2021b; Franzoi et al., 2022). After birth, calves were immediately separated from their dams, and the first colostrum was sampled within the first 6 h after calving. The time window was selected considering results from Conneely et al. (2013), who reported that colostrum produced

later than 9 h after parturition had a statistically lower IgG concentration compared with colostrum produced earlier. To avoid presence of hyperimmune colostrum, all the cows included in this study were not vaccinated before calving against common agents such as coronavirus, rotavirus, and *Escherichia coli* (Dunn et al., 2017). The selected farms followed an intensive farming system, with freestall barn, total mixed ration administration, twice-a-day milking, and no access to pasture.

The sampling protocol (Costa et al., 2021a,b, 2022) allowed 120 mL of colostrum from each cow to be obtained. Briefly, sample collection was carried out under farmers' responsibility using plastic sterile tubes with indication of the cow's ID, parity, and calving date (SMIPA srl, Vicenza, Italy). Samples were subsequently stored on farm at -20°C and, by maintaining the cold chain, they were periodically transferred to the laboratory of Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova (Legnaro, Italy), where they were kept at freezing temperature until further analyses.

Gross Composition Analysis and Immunoglobulin Quantification

After thawing overnight at 4°C , 40 mL of each sample was lyophilized to measure protein and fat content. The first was quantified by Kjeldahl method 991.20 (AOAC International, 2000), whereas the second was determined according to Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten (VDLUFA) VI C15.2.1 method (VDLUFA, 2013). Lactose quantification was carried out from an aliquot of colostrum by using a Jasco high-performance liquid chromatograph equipped with an Aminex HPX 87H column (300 mm $\times$ 7.8 mm, Bio-Rad), and data were interpreted using the ChromNAV software (Version 2.0, Jasco) where the final concentration of lactose was calculated.

The concentration of IgG, IgA, and IgM was assessed with RID as widely described by Costa et al. (2021b) using Bovine Ig RID Kit purchased from Triple J Farms (Bellingham, WA). An overview of the number of samples available is given in Supplemental Table S1 (<https://doi.org/10.7910/DVN/RXBC1Z>; Goi et al., 2023). To fall within the detection range of the RID assay, colostrum aliquots were diluted with deionized purified water: 1:5 (vol/vol) for IgG and 1:3 (vol/vol) for IgA and IgM. For the calculation of the punctual concentration of the target components, the standard curve obtained for each plate ($n = 21$ samples) from the 3 reference sera/standards provided by the manufacturer (Triple J Farms, Bellingham, WA) was used. The RID repeatability, described in detail by Costa et al. (2021b), has been tested by means of the in-

tra- and interassay coefficients of variation (CV). The first was tested using 4 colostrum samples analyzed in quintuplicate by a single operator: the overall CV was calculated as the average of the individual coefficients of the 4 samples measured in quintuplicate and was equal to 7.56, 2.46, and 3.03% for IgG, IgA, and IgM, respectively (Costa et al., 2021b). The interassay CV was calculated to check for potential analytical errors using the ring diameter of the 3 standards (concentrations can be found in Costa et al., 2021b) measured in 5 different plates. The average CV of the 5 plates was calculated separately for each concentration (high, medium, and low) within each parameter (IgG, IgA, and IgM) by averaging the coefficients. The resulting interassay CV was 4.63, 4.27, and 2.46% for IgG, IgA, and IgM, respectively.

MIRS Prediction Models

Contextually to RID analysis, a representative amount of the thawed colostrum was diluted (1:1, 25 mL in 25 mL) in pure water. Dilution was necessary before spectra collection to reduce the density of the matrix and avoid mechanical issues due to high density or clots. Spectra (Supplemental Table S1) were recorded using the Milkoscan 7 RM spectrometer of the ARAV official milk laboratory (Vicenza, Italy). The benchtop instrument operates at room temperature (20°C) in a wavelength range between 5,011.54 and 925.92 cm^{-1} every 3.85 cm^{-1} , for a total of 1,060 data points recorded, which were collected using FOSS Integrator software (FOSS Electric A/S, Hillerød, Denmark). Spectral data, coupled with the abovementioned RID reference analyses, were used to predict the concentration of IgG, IgA, IgM, and total Ig through the PLS procedure (SAS v. 9.4; SAS Institute Inc., Cary, NC). In addition to Ig, prediction models were also trained for the gross composition traits to obtain MIRS prediction of protein, fat, and lactose.

Before building the model, spectra were independently subjected to standard normal variate (SNV) scatter correction, one of the most popular pre-processing treatments of MIRS data (De Marchi et al., 2014). The SNV aims to normalize the spectral data reducing baseline shifting or tilting due to nonspecific scattering of radiation (Manfredi et al., 2018). In fact, to obtain robust prediction models, pre-treatments have great importance to reduce phenomena that can cause a deviation from the linear relation from spectrum and reference value such as scatter from particulates, molecular interactions, and changes in sample size (Rinnan et al., 2009). Spectral regions with low signal-to-noise ratio due to water absorption have been discarded according to the producer (FOSS Electric A/S). Last,

spectra presenting a Mahalanobis distance >3 from the average spectrum were considered as outliers and removed. The final data set comprised 338 wavenumbers, expressed into absorbance, in the intervals 2,970.7 to 2,507.7 cm^{-1} , 1,929 to 1,716.8 cm^{-1} , and 1,581.8 to 964.5 cm^{-1} for each sample. After spectrum pretreatments, the complete data set was randomly split in a calibration set representing 75% of total samples and in a validation set (remaining 25%), both of which had similar mean and standard deviation of the target trait (IgG, IgA, IgM, total IgG, and gross composition traits) and which were representative of all herds and parities. For each trait, a first PLS regression analysis was performed on the calibration subset by using a self-built macro (SAS software v. 9.4, SAS Institute Inc.) that trained the model with a 5-fold cross-validation. The other subset was used to assess the final performance of the developed calibration model in external validation. To avoid overfitting, the number of latent variables included in the PLS were those that minimize the root mean square error calculated at each iteration of cross-validation (Franzoi et al., 2022). For each model, the standard error of cross-validation (SE_{CV}) was calculated and reference value outliers, detected as samples whose prediction deviated more than $\pm 2.5 \text{ SE}_{\text{CV}}$ from the reference value, were excluded. The methodology of PLS regression analysis followed by outliers' elimination was iterated 2 additional times consecutively and the variable importance in projection (VIP) scores of the last round of PLS were kept. Residual normality of prediction equations were checked, and a *t*-test was performed using the TTEST procedure of SAS to assess that the bias, calculated as the average difference between predicted and reference values, did not statistically differ from zero. The best prediction equation was identified considering the coefficient of determination of cross- (R^2_{CV}) and external validation (R^2_{V}), and residual predictive deviation (RPD) in external validation.

Diagnostic Accuracy of MIRS-Predicted Traits

Receiver operating characteristic (ROC) curves were used to determine specificity (true negative cases), sensitivity (true positive cases), and overall discriminant ability of protein content, IgG, and total Ig predicted through MIRS in external validation. In particular, quality of samples was classified according to the RID IgG concentration as follows: $<$ and ≥ 70 g/L for low and high quality, respectively. The area under the curve was the parameter used to evaluate the discriminant ability of the proxy (Šimundić, 2009). The data points of ROC curves and calculation of sensitivity and specificity were obtained in SAS software v. 9.4 (SAS Insti-

tute Inc.) through a customized script that included the LOGISTIC procedure. Subsequently, the Youden index was calculated to identify the best cut-off value for each trait, namely protein content (predicted via MIRS and measured via gold standard) and MIRS-predicted IgG, IgA, IgM, and total Ig.

RESULTS AND DISCUSSION

The samples used were derived from an experiment carried out between 2019 and 2020. Part of the results from this trial on phenotypic and genetic variability of Ig as well as on near-infrared spectroscopy applications and use of refractometers have already been published (Costa et al., 2021a,b, 2022; Franzoi et al., 2022). For the purpose of the present investigation, the main inclusion criterion was the presence of the mid-infrared spectrum (Supplemental Table S1). Out of the 714 samples originally collected, only those belonging to purebred Holstein animals calving between spring 2019 and spring 2020 and with both mid-infrared spectra and reference available were kept. In some cases, in fact, one or more aliquots were lost and the volume was insufficient (<25 mL) for mid-infrared spectra acquisition (Supplemental Table S1). The number of samples with matching reference measurement and spectrum is reported in Table 1 for each trait. The average parity, ranging from 1 to 8, differed thereby according to the trait considered (i.e., from 2.39 for IgA to 2.48 for IgG and IgM).

Descriptive Statistics

Descriptive statistics of colostrum Ig isotypes and composition traits postediting are summarized in Table 1. The final number of records ranged from 455 (IgA) to 577 for lactose content. The most variable Ig fraction was IgA with a coefficient of variation close to 60% (Table 1), whereas, among composition traits, fat content exhibited the greatest variability (65.94%). According to the averages observed, approximately 91% of total Ig is composed by the G fraction, followed by fractions M and A, which each comprised 5 and 4% of the total Ig, respectively. As expected, there was large variability in the colostrum composition, even if the time window for sampling was narrow (0 to 6 h after calving) compared with other studies where colostrum was sampled as late as 24 h postpartum. The rapid change in both the chemical and the physical properties of bovine colostrum has been widely described in literature, particularly in the case of IgG (Bielmann et al., 2010; McGrath et al., 2016). For instance, Baumrucker and Bruckmaier (2014) reported a wide cow-to-cow variation of the subtype IgG1 (11.8 to 74.2 mg/

Table 1. Overview of descriptive statistics of colostrum¹ Ig isotypes and composition traits measured with the reference method

Trait	N	Mean	SD	CV, %	Range
Ig, g/L					
IgG	531	93.54	33.87	36.21	9.22–198.90
IgA	455	4.56	2.59	56.80	0.13–13.89
IgM	522	5.06	2.37	46.84	0.35–11.39
Total	458	102.82	35.04	34.08	19.67–196.86
Protein, %	559	14.71	3.51	23.86	4.55–25.22
Fat, %	557	4.61	3.04	65.94	0.12–14.95
Lactose, mg/100 mg	577	2.36	0.51	21.61	0.74–4.06

¹Sampled within 6 h from calving.

mL) and the range observed by Kessler et al. (2020) for RID IgG (12.7 to 185.3 mg/mL) is similar to ours (9.2 to 198.8 g/L). Overall, the concentrations obtained also mirrored those reported by Ceniti et al. (2019) for Simmentals (0.1 to 199.0 g/L) and by Biemann et al. (2010) for Holsteins (22.4 to 196.9 g/L). Concentrations of IgA and IgM were similar to those obtained in Holstein cows by Gomes et al. (2011). The composition of colostrum is highly variable due to numerous factors, including individual cow, breed, parity, calving season, pre-partum nutrition, dry period length and management, and sampling time postpartum (McGrath et al., 2016; Costa et al., 2023).

Of all the samples with the RID IgG value available, 9% were characterized by a suboptimal IgG concentration (i.e., below 50 g/L). According to McGrath et al. (2016) and Godden et al. (2019), this value represents the threshold to discriminate between low- and high-quality colostrum.

Descriptive statistics for fat, protein, and lactose are consistent with Morrill et al. (2012) who reported average values of 5.6, 12.7, and 2.9%, respectively, and with Kehoe et al. (2007) whose averages were 6.7, 14.9, and 2.5%, respectively. Compared with such authors, fat concentration quantified in the present study was lower, likely due to the analytical method used. Considering the variability, the high CV obtained are consistent with results of Morrill et al. (2012) and Kehoe et al. (2007) who reported a very wide range for both fat (1.0–21.7% and 2.0–26.5%, respectively) and protein content (2.6–20.5% and 7.1–22.6%, respectively). Values reported by Kehoe et al. (2007) are referred to cows fully milked out within 4 h of calving, suggesting that the fat variability is not related to the milking procedure (e.g., empty milking or not) but more likely to the sampling time after calving or other cows' physiological reasons.

Predictive Ability

As in milk, the reference methods to measure colostrum quality are expensive, laborious, and hardly

implementable for large-scale acquisition of phenotypes. Previous studies have already highlighted the ability of near-infrared spectroscopy to predict some traits related to colostrum composition, including IgG (Navrátilová et al., 2006; Rivero et al., 2012; Franzoi et al., 2022). Elsohaby et al. (2018) developed a MIRS model for colostrum IgG concentration, but in this study for the first time MIRS has been used to develop and validate prediction models for the 3 Ig fractions (G, A, and M) and gross composition traits. These may be features of interest in contexts where precise quantification is needed, for example, to develop precision feeding strategies (e.g., for calves with poor vitality at birth who may require a particularly excellent colostrum quality), to assess payment system based on the Ig level, and to have phenotypes available for breeding purposes (Costa et al., 2023).

For all the traits investigated in the current study, the performance of the best MIRS prediction model developed in cross- and external validation are reported in Table 2. In the case of Ig, the samples identified as outliers and excluded from the calibration data set were below 5% and the LV ranged from 3 to 10. The most excellent model in terms of variance explained in cross-validation (Karoui et al., 2006) was the one developed for IgG ($R^2_{CV} = 0.92$), followed by the one for total Ig concentration ($R^2_{CV} = 0.90$). When applied on an external set of samples, the models for IgG and total Ig content showed promising performances ($R^2_V > 0.82$) and the RPD was >2.3 , indicating the feasibility of using the prediction equations for rough screening of samples (Williams, 2014). The prediction model developed by Franzoi et al. (2022) for colostrum IgG using near-infrared spectroscopy has a lower R^2_V (0.76) with PLS algorithm, but almost similar coefficient of determination (i.e., 0.83) to that reported in the present study for IgG when applying simulated annealing PLS. The prediction model of IgM and IgA did not reach satisfactory performance (Table 2), as similarly observed by Franzoi et al. (2022) under near-infrared spectroscopy. Through beef and dairy cow colostrum samples, Elsohaby et al. (2018) obtained promising pre-

Table 2. Fitting statistics¹ of modified partial least square regressions in 5-fold cross-validation and external validation for Ig isotypes of colostrum²

Ig	n	Outliers, %	LV	RMSE _{CV}	R ² _{CV}	n _{ext}	RMSE _V	R ² _V	RPD
IgG, g/L	383	4.0	10	9.53	0.92	132	13.39	0.84	2.49
IgA, g/L	329	3.8	5	1.68	0.45	113	2.08	0.33	1.22
IgM, g/L	384	2.0	3	1.65	0.49	130	1.94	0.33	1.21
Total, g/L	328	4.7	8	10.31	0.90	114	14.59	0.83	2.38

¹n = number of samples used to perform the calibration after the outliers' elimination; LV = number of latent variables; RMSE_{CV} = root mean square error in cross-validation; RMSE_V = root mean square error in external validation; R²_{CV} = coefficient of determination in cross-validation; n_{ext} = number of samples used to perform the external validation; R²_V = coefficient of determination in external validation; RPD = residual predictive deviation calculated as standard deviation of reference data (validation) to standard error of predictions.

²Sampled within 6 h from calving.

dictability for IgG with MIRS using a device working in a smaller range of wavelength (4,000 and 400 cm⁻¹) compared with that of the present study. However, the sampling protocol adopted by Elsohaby et al. (2018) apparently does not allow to fairly compare their model with ours. In particular, authors did not report the time window considered for sampling, precluding direct comparison of the reference and predicted data (God-den et al., 2019).

The scatter plots of measured versus predicted Ig isotypes and total Ig for the external validation set, and their associated R²_V, are reported in Figure 1. The mean and the range of measured and predicted traits are reported in Table 3 and demonstrate that MIRS predictions are in line with the gold standard RID even though characterized by a slightly lower variability. Indeed, within a given trait, the coefficient of variation was larger for the reference compared with its predicted counterpart.

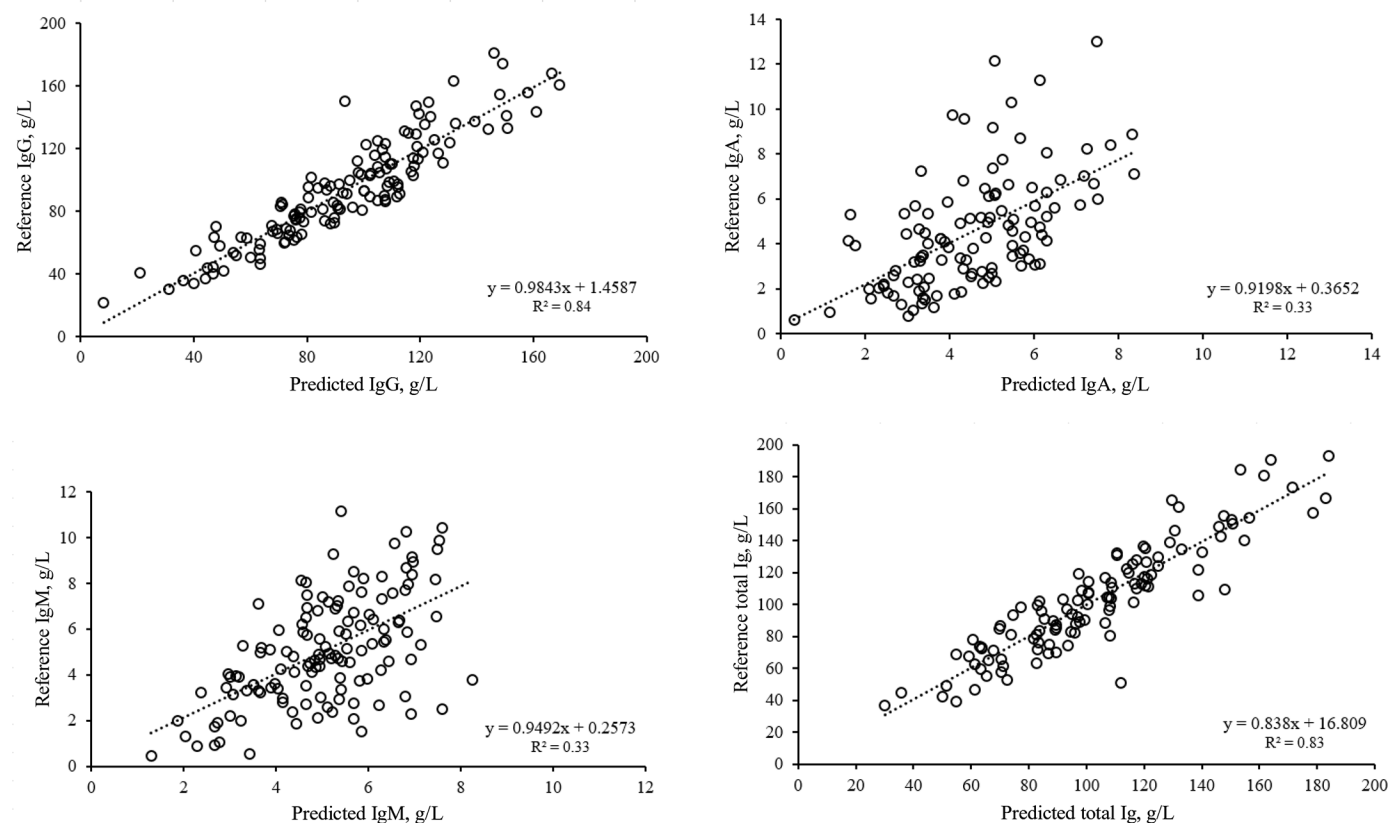
**Figure 1.** Plot of predicted versus reference values of the Ig isotype concentration and concentration of total Ig of colostrum. Sampled within 6 h from calving; external validation set.

Table 3. Descriptive statistics of the Ig isotypes of colostrum¹ determined via radial immunodiffusion (RID) and predicted via mid-infrared spectroscopy (MIRS) in the calibration and the validation sets

Ig	Determination	Data set	n	Mean	SD	CV, %	Range
IgG, g/L	RID	Calibration	399	93.55	34.11	36.46	9.22–198.90
		Validation	132	93.50	33.28	35.59	21.48–181.10
	MIRS	Calibration	383	92.40	31.55	34.15	7.31–177.64
		Validation	132	93.51	30.98	33.13	8.06–169.35
IgA, g/L	RID	Calibration	342	4.56	2.61	57.24	0.13–13.89
		Validation	113	4.54	2.53	55.73	0.61–13.01
	MIRS	Calibration	329	4.34	1.57	36.18	0.00–8.65
		Validation	113	4.54	1.59	35.02	0.32–8.37
IgM, g/L	RID	Calibration	392	5.06	2.37	46.84	0.35–11.39
		Validation	130	5.07	2.36	46.55	0.47–11.17
	MIRS	Calibration	384	5.06	1.65	32.61	0.37–9.19
		Validation	130	5.07	1.42	28.01	1.29–8.25
Total, g/L	RID	Calibration	344	102.77	35.18	34.23	19.67–196.86
		Validation	114	102.97	34.77	33.77	37.02–192.66
	MIRS	Calibration	328	101.33	31.73	31.31	16.68–186.89
		Validation	114	103.10	32.07	31.11	30.04–184.16

¹Sampled within 6 h from calving.

Table 4 reports the fitting statistics of developed calibration models for protein, fat, and lactose content. The reference outliers (about 5%) were excluded before development of the MIRS model of protein and lactose, and for both traits the developed model was quite satisfactory in terms of R^2_V , being 0.89 and 0.86 for protein and lactose, respectively. Due to the high variability of fat content already discussed (Table 1), the number of outliers removed was close to 10%. Nevertheless, even the R^2_V for fat indicated that the developed prediction model can still be considered sufficient for screening and to approximate quantitative prediction (Williams, 2007; Grelet et al., 2021). In support of this, Figure 2 shows the linear relationship between the measured and predicted values of colostrum gross constituents in the external validation set. Navrátilová et al. (2006) obtained similar R^2_{CV} for colostrum protein and lactose content prediction models using near-infrared spectroscopy; with regard to fat, however, the model developed was characterized by a greater R^2_{CV} compared with the present study. However, it was not possible to compare the R^2_V as no external validation was performed. Overall, the RPD value for the developed prediction models in the current study indicates a promising feasibility

of MIRS to assess the level of protein and lactose in bovine colostrum. Further implementation of the models, coupled with the use of other algorithms (Gottardo et al., 2015; Frizzarin et al., 2021), could likely result in an increased predictive ability, particularly for fat content. The range of measured and predicted values is reported in Table 5 for both the calibration and the validation sets; as mentioned for the Ig fractions, MIRS predictions fell within the range of measured traits even if they tended to be slightly less variable. In the external validation set, for example, the minimum protein and fat content was smaller than the minimum of their respective reference. With regard to lactose content, only small differences were seen (Table 5). In particular, in external validation, the range of the gold standard (1.16–4.03 mg/100 mg) and the range of MIRS (1.47–4.17 mg/100 mg) were almost the same. The same is valid for the calibration set (Table 5), whose ranges were 0.74–4.06 (gold standard) and 0.97–4.24 (MIRS). Overall, such findings confirm that the models provided robust predictions that fell within the range of values we expected. To assess a punctual determination of constituents for commercial purpose/industrial use, however, we recommend increasing the sample size

Table 4. Fitting statistics¹ of modified partial least square regressions in 5-fold cross-validation and external validation for the colostrum² gross composition traits

Trait	n	Outliers, %	LV	RMSE _{CV}	R^2_{CV}	n_{ext}	RMSE _V	R^2_V	RPD
Protein, %	399	5.0	12	0.81	0.94	139	1.16	0.89	2.97
Fat, %	374	10.5	5	0.78	0.92	139	1.57	0.74	1.96
Lactose, mg/100 mg	410	5.3	10	0.14	0.91	144	0.19	0.86	2.63

¹n = number of samples used to perform the calibration after the outliers' elimination; LV = number of latent variables; RMSE_{CV} = root mean square error in cross-validation; RMSE_V = root mean square error in external validation; R^2_{CV} = coefficient of determination in cross-validation; n_{ext} = number of samples used to perform the external validation; R^2_V = coefficient of determination in external validation; RPD = residual predictive deviation calculated as standard deviation of reference data (calibration) to standard error of predictions.

²Sampled within 6 h from calving.

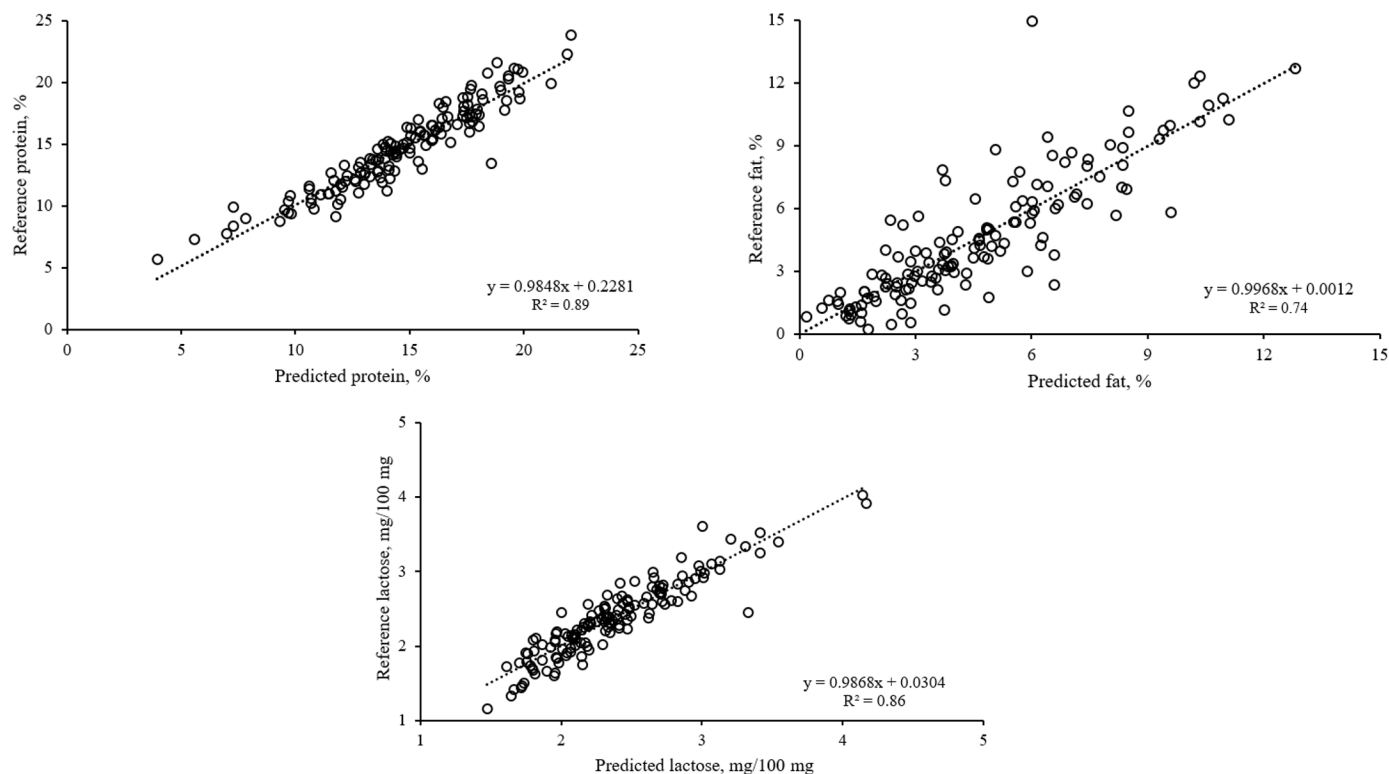


Figure 2. Plot of predicted versus reference values of the gross composition trait concentrations of colostrum. Sampled within 6 h from calving; external validation set.

and variability (e.g., breed, country, farming system) to improve the representativeness and robustness of the calibration sets.

VIP Scores

To compare the spectral regions significant for predicting IgG, total Ig, and protein content, we extrapolated the VIP scores (Figure 3) to identify the most important wavenumbers (i.e., those with a VIP score

greater than 1; Caponigro et al., 2023). In fact, the VIP score measures the significance of a component in regression. As expected, the significant regions of the 3 traits overlaps in some spectral wavenumbers. With regard to IgG and total Ig, this was expected since the former accounts for the major percentage of the latter; VIP scores presented peaks for IgG which are always included by those of total Ig. The peaks are located in regions known to be important for protein compounds (i.e., around $1,550\text{ cm}^{-1}$ due to C–N and

Table 5. Descriptive statistics of colostrum¹ gross composition traits determined via gold standard² and predicted via mid-infrared spectroscopy (MIRS) in the calibration and the validation sets

Trait	Determination	Data set	n	Mean	SD	CV, %	Range
Protein, %	Gold standard	Calibration	420	14.71	3.53	24.00	4.55–25.22
		Validation	139	14.71	3.45	23.45	5.71–23.80
	MIRS	Calibration	399	14.79	3.32	22.45	4.69–23.77
		Validation	139	14.71	3.30	22.43	3.94–22.07
Fat, %	Gold standard	Calibration	418	4.60	3.04	66.09	0.12–14.95
		Validation	139	4.63	3.07	66.31	0.24–14.93
	MIRS	Calibration	374	4.38	2.70	61.64	0.29–14.10
		Validation	139	4.64	2.66	57.33	0.00–12.83
Lactose, mg/100 mg	Gold standard	Calibration	433	2.36	0.51	21.61	0.74–4.06
		Validation	144	2.37	0.51	21.10	1.16–4.03
	MIRS	Calibration	410	2.36	0.45	19.07	0.97–4.24
		Validation	144	2.37	0.47	19.83	1.47–4.17

¹Sampled within 6 h from calving.

²Protein, fat, and lactose content were determined via Kjeldahl, base hydrolysis plus accelerated solvent extraction, and HPLC, respectively.

N–N stretching; Grelet et al., 2015) and N–H bending deformation (Ribadeau-Dumas and Grappin, 1989). Moreover, Grelet et al. (2015) and Ribadeau-Dumas and Grappin (1989) stated that another absorption band of the peptide bond exists around $1,650\text{ cm}^{-1}$. Soyeurt et al. (2012) reported regions between $1,700$ and $1,500\text{ cm}^{-1}$ and from $1,450$ to $1,200\text{ cm}^{-1}$ to be associated with protein absorption bands. However, the most significant wavenumber identified also covered a relevant range for the carbohydrate functional group ($1,200$ to 900 cm^{-1} ; Caponigro et al., 2023), lactose content ($1,250$ to $1,000\text{ cm}^{-1}$) and fat content ($1,450$ to $1,390\text{ cm}^{-1}$; Grelet et al., 2015). These similarities confirm that antibodies represent most of the protein contained in the first colostrum. However, in terms of dissimilarities, certain wavenumbers in some regions were useful to predict protein content but not Ig, and vice versa (Figure 3). This indicates that, despite being highly correlated, the IgG and the total protein content cannot be considered as the same trait.

Diagnostic Accuracy

Sensitivity and specificity of MIRS-predicted IgG and total Ig are graphically presented in Figure 4 and refer to the external validation set. In the case of IgG ($n = 132$), the AUC was excellent (0.978) and not far from the AUC of total Ig (0.918; $n = 114$). These values denote an excellent discriminant ability of the 2 MIRS-predicted traits (Šimundić, 2009). We also tested the discriminant ability of measured and predicted protein content (Figure 5); their AUC were very satisfactory, equaling to 0.952 and 0.957 ($n = 127$), respectively. Overall, these results are presented as complementary information about the model performance to classify colostrum samples according to their quality. Based on the false positives and false negatives observed (Table 6), MIRS-predicted traits, namely IgG, total Ig, and protein content, correctly classified 90.1%, 84.2%, and 92.2% of the total samples. In the case of protein content measured via gold standard, this percentage was 92.9%. True positives, however, tended to be classified more accurately than true negatives (Table 6), as suggested by the non-zero amount of false positives, i.e., negative samples (low-quality) incorrectly classified as positives (high-quality). The optimal cut-off value, the point on the ROC curve that maximizes sensitivity and specificity (Table 6), was found at 75.4 g/L in the case of IgG and at 95.09 g/L in the case of total Ig (Figure 4). Finally, the cut-offs for MIRS-predicted protein content and protein content determined through the gold standard were 13.32 and 12.65%, respectively, indicating that samples with a MIRS-predicted protein content of 13.32% have to be considered of low quality.

Ideally, colostrum with IgG greater than 50 g/L should be used for the first calf's meal as the absolute amount of antibodies delivered would be sufficient if a conventional volume ($\sim 4\text{ L}$) is administered in the first 12 h of life (Buczinski and Vandeweerdt, 2016; McGrath et al., 2016).

We tested the ability to discriminate samples base on the quality level by using 70 g/L of IgG as a threshold, as the conventional cut-off (50 g/L) seemed too strict, with a very limited number of samples ($n = 12$) in the low-quality group. The 50 g/L would lead to very high AUC (0.979), a cut-off of 67.62 g/L , and 95.5% of samples classified correctly (data not shown). In particular, 117 high-quality samples would be the true positives and 9 low-quality samples the true negatives; the remaining amount would be given by the 3 false positives and the 3 false negatives (data not shown).

Practical Considerations

The IgG and gross composition determination via MIRS may be useful to standardize pooled colostrum at industry level (Franzoi et al., 2022), to collect the narrow-sense quality phenotypes at the individual level for selective breeding (i.e., IgG), or to further aid farmers in setting up an internal farm colostrum bank (Costa et al., 2021a, 2022). The list of items analyzable by MIRS-based instruments of milk laboratories or dairy plants may be potentially extended with colostrum. Considering that this secretion is only produced at calving, just 1 sample/year would be analyzed for each lactating animal. This number can be even lower if exclusively colostrum of elite/selected cows is taken (e.g., for colostrum bank or for selling). In most of the countries with a milk recording service, farmers already pay a fee to analyze the milk of their cows 9/10 times per year. Given these considerations and bearing in mind the increasing importance of colostrum in different research fields (Costa et al., 2023), we expect the cost of colostrum MIRS analysis to be almost negligible for farmers in the near future. Devices like refractometers and colostrometers provide indirect measure of total solids content and the relationship with the IgG is strongly time-dependent (h from parturition), making the measurements obtained from different farmers and tools not comparable and often imprecise. The MIRS prediction of colostrum composition, instead, requires the same personnel and instruments already in use for the official milk analyses worldwide, under the specific rules and standard guidelines of the International Committee for Animal Recording (ICAR), without any need of additional resources. Farmers willing to analyze the colostrum of all or certain cows only need to freeze or refrigerate the bottles/tubes and then deliver them to

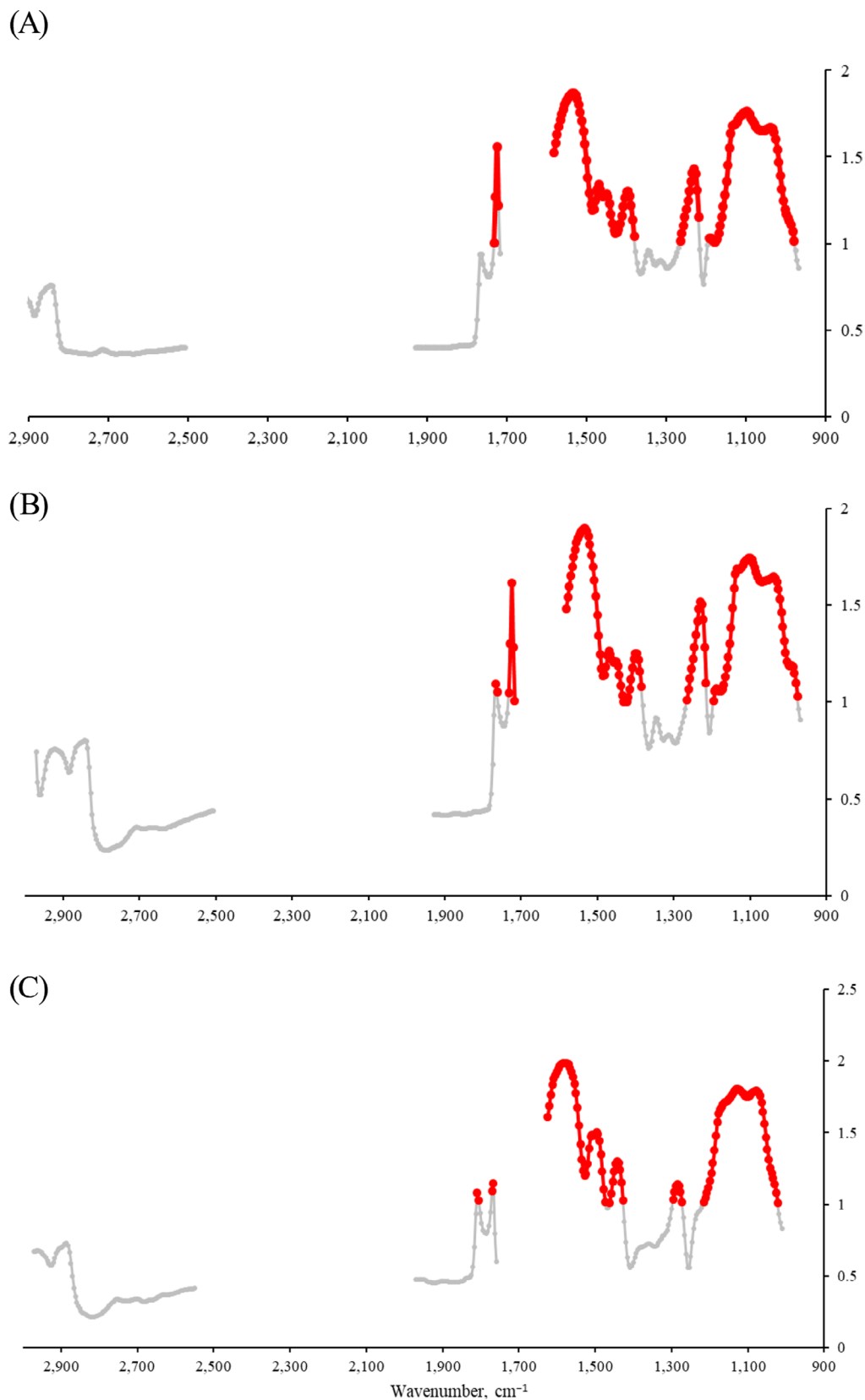


Figure 3. Total wavenumbers used for prediction of (A) IgG concentration, (B) total Ig concentration, and (C) protein content with identification of the most important wavenumbers (red dots).

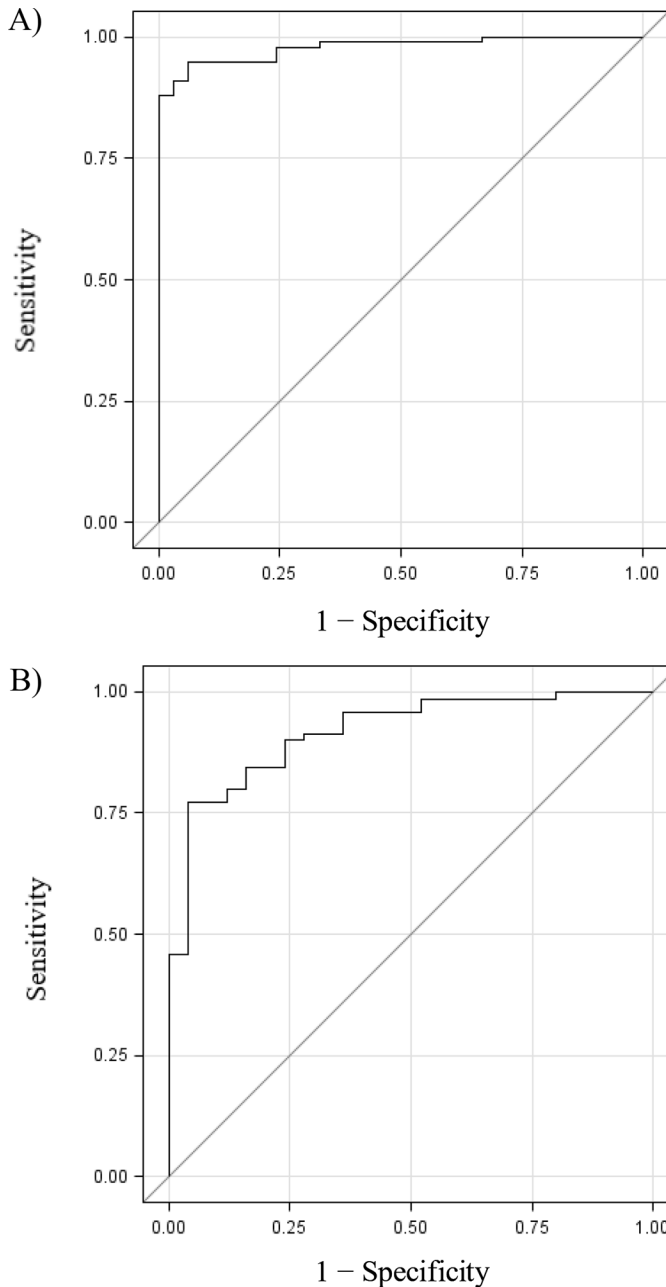


Figure 4. Graphical output of the receiver operating characteristic analysis to assess the discriminant ability of infrared-predicted (A) IgG concentration ($n = 132$) and (B) total Ig concentrations ($n = 114$) of samples present in the external validation set with the gold standard IgG concentration available. Low and high quality: $<$ and ≥ 70 g/L of IgG (determined via gold standard), respectively.

the laboratory, possibly with the support of the person in charge of the monthly official milk analysis.

The MIRS analysis can allow identification of the top-quality colostrum samples that could be used when precision feeding strategies are needed to ensure a suc-

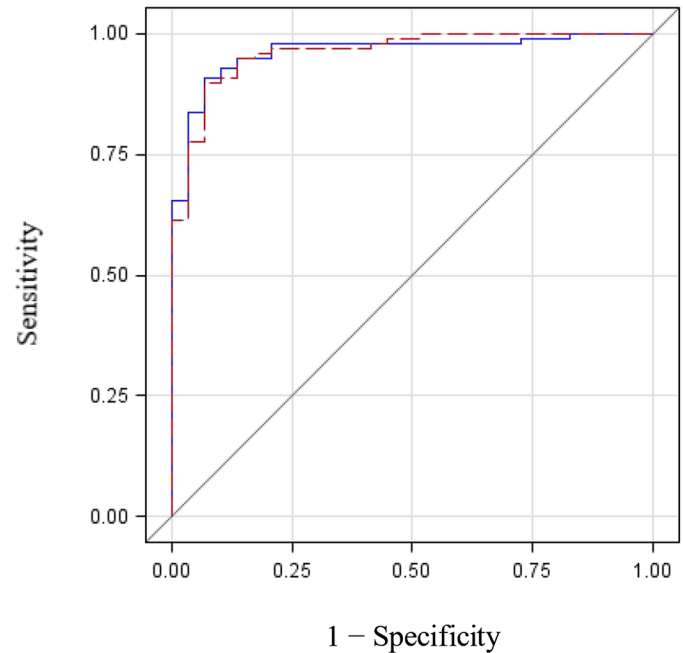


Figure 5. Graphical output of the receiver operating characteristic analysis to assess the discriminant ability of protein content measured via gold standard (blue solid line) and predicted via infrared spectroscopy (red dashed line) in the 127 samples present in the external validation set with gold standard IgG concentration available. Low and high quality: $<$ and ≥ 70 g/L of IgG (determined via gold standard), respectively.

cessful transfer of antibodies to calves born with low vitality and weak suckling.

Finally, it is important to highlight that more flexible MIRS models can be developed in future for commercial dissemination using colostrum collected even after 6 h from parturition for calibration (e.g., from 0 to 12 h after calving).

CONCLUSIONS

In the present study, we attempted to predict Ig concentration and gross composition of first colostrum of dairy cows using MIRS, a technology that requires less than 30 mL of sample for spectra collection and handles more than 200 samples per hour. This technology showed good to excellent performance in predicting concentration of colostrum antibodies and traditional composition traits in cross-validation and demonstrated a promising and good predictive ability in external validation. In particular, the performance was outstanding in the case of IgG concentration, the key parameter for the definition of colostrum quality in cattle. In addition, we demonstrated that through MIRS-predicted IgG and protein content it is possible to accurately identify low-quality samples by using specific cut-offs: 75.40 g/L

Table 6. Confusion matrix for the discriminant ability of the MIRS-predicted traits and gold standard protein content of samples in the external validation set with the gold standard IgG concentration available¹

Predicted classification	Actual classification ²		AUC	Cut-off
	Positive	Negative		
MIRS				
IgG concentration (n = 132)			0.978	75.40 g/L
Positive	92%	17%		
Negative	8%	83%		
Total Ig concentration (n = 114)			0.918	95.09 g/L
Positive	88%	27%		
Negative	12%	73%		
Protein content (n = 127)			0.961	13.32%
Positive	94%	15%		
Negative	6%	85%		
Gold standard				
Protein content (n = 127)			0.962	12.65%
Positive	94%	12%		
Negative	6%	88%		

¹The area under the curve (AUC) is specified together with the best cut-off identified. Low and high quality: < and ≥70 g/L of IgG (determined via gold standard), respectively. MIRS = mid-infrared spectroscopy.

²The percentage of true positives and the percentage of true negatives are the first and the second element in the diagonal, respectively.

and 13.32%, respectively. Considering the importance of having punctual IgG concentration for management, genetic, and analytical purposes, this study paves the way toward the implementation of dedicated models for routine MIRS analysis of bovine colostrum. As for milk, MIRS represents a valid and low-cost phenotyping tool for colostrum to be used for (1) genetic purposes on the animal side and (2) for quality assessment at farm and industry level (food and pharma).

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**Minerals and essential amino acids of bovine colostrum: Phenotypic variability
and predictive ability of mid- and near-infrared spectroscopy**

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Minerals and essential amino acids of bovine colostrum: Phenotypic variability and predictive ability of mid- and near-infrared spectroscopy

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ABSTRACT

Colostrum quality and volume are fundamental for calves because it is the primary supplier of antibodies and the first source of energy, carbohydrates, lipids, proteins, minerals, and vitamins for the newborn. Assessing the detailed composition (i.e., AA and mineral content) of bovine colostrum (BC) on-line and at a reasonable cost would help dairy stakeholders such as farmers or veterinarians for precision feeding purposes and industries producing preparations containing BC such as foodstuff, supplements, and medicaments. In the present study we evaluated mid- (MIRS) and near-infrared spectroscopy (NIRS) prediction ability for AA and mineral composition of individual BC. Second, we investigated the major factors affecting the phenotypic variability of such traits also evaluating the correlations with the Ig concentration. Results demonstrated that MIRS and NIRS were able to provide sufficiently accurate predictions for all the AA. The coefficient of determination in external validation (R^2_v) fell, in fact, within the range of 0.70 to 0.86, with the exception of Ile, His, and Met. Only some minerals reached a sufficient accuracy (i.e., Ca, P, S, and Mg; $R^2_v \geq 0.66$) using MIRS, and also S ($R^2_v = 0.87$) using NIRS. Phenotypically, both parity and calving season affected the variability of these BC composition traits. Heifers' colostrum was the one with the greatest concentration of Ca and P, the 2 most abundant minerals. These minerals were however very low in cows calving in summer compared with the rest of the year. The pattern of AA across parities and calving season was not linear, likely because their variability was scarcely (or not) affected by these effects. Finally, samples characterized by high IgG concentration were those presenting on average greater concentration of AA. Findings suggest that infrared spectroscopy has the potential to be used to predict certain AA and minerals, outlining

the possibility of implementing on-site analyses for the evaluation of the broad-sense BC quality.

Key words: colostrum quality, amino acids, dairy cow, dairy industry

INTRODUCTION

Colostrum management is a fundamental aspect to account for calves' survival and health in dairy farms, as a suboptimal quantity or quality can affect the success of passive transfer of immunity in newborns. In bovines like in other livestock species, in fact, Ig are solely acquired through passive absorption via colostrum (Godden, 2008). The farmers' goal is to achieve serum concentrations of IgG in calves >10 g/L between 24 and 48 h after birth. In addition to being a source of fundamental antibodies for calves, colostrum remains the first source of energy, carbohydrates, lipids, proteins, minerals, and vitamins for the neonates. The presence of EAA and minerals makes the first meal of calves crucial for health and functionality of the organism (Blum and Hammon, 2000).

Certain AA are essential for proteins synthesis and have a fundamental role for the growth and development of the organism. This is particularly the case in calves, as the synthesis of protein is greater in the early postnatal stage of life than in any other period (Zanker et al., 2000). The AA also promote immunological activity and, to a lesser extent, are precursors for the synthesis of some metabolites (Li et al., 2020). Finally, they can be used to produce metabolic energy when oxidized to carbon dioxide (NRC, 2001).

Major minerals such as Ca, P, S, and K are fundamental for the formation of skeletal tissue, bone maintenance, biosynthesis of specific molecules, acid-base regulation, and nerve impulse transmission. Minor elements, namely Na, Mg, Zn, and Fe, instead, are present in a lower concentration but still have some key roles. As a matter of fact, Na, Mg, Zn, and Fe are either involved in the creation of electric gradients for nutrient transport, in muscle function and bone formation, or are a component of the heme which is found in hemoglobin and myoglobin (NRC, 2001).

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Knowing the detailed colostrum composition, in addition to the narrow-sense quality given by the IgG concentration (Costa et al., 2021, 2023), can be of interest even after the farm gate. This means that fine colostrum composition is interesting not only for precision feeding and for veterinarian use on the farm. In fact, bovine colostrum (BC) is becoming a popular ingredient for humans, several livestock species, and pets, meaning that there might be an industrial interest behind the assessment of fine composition traits such as minerals and AA. Assessing the BC composition at a reasonable cost and on-line would definitely boost the production and the safety of foodstuff, supplements, and medicaments containing BC and entering in the market for human use. Existing methods for AA and mineral determination in milk and dairy products are in most of the case gold standards (i.e., accurate, but time-consuming and expensive, methods). In the light of these considerations, infrared spectroscopy—already in use for analysis of foodstuff worldwide—may be adopted for BC fine composition assessment if the prediction accuracy permits it. The cost-effectiveness, power, and ease of implementation of infrared spectroscopy are widely known in the dairy sector: mid-infrared spectroscopy (MIRS) is used for official analysis of liquid milk (De Marchi et al., 2014), and near-infrared spectroscopy (NIRS) allows analysis of solid or semi-solid mediums for phenotyping purposes and for determination of quality traits (De Marchi et al., 2018). Recently, both MIRS and NIRS have shown promising prediction accuracy for colostral IgG in Holstein cows. A limited number of studies explored traits such as AA and minerals of BC using a large number of samples and to the best of authors' knowledge, this is the first time infrared spectroscopy has been used to predict such features in BC on a large data set. It is the authors' belief that—whenever accurate—prediction models could be meaningful and useful within the dairy chain and sector at different levels, particularly in contexts such as the food industry and pharmaceutical sector, to assess rapidly and at a low cost the main compositional properties. Out of all stakeholders of the dairy industry, manufacturers using BC as an industrial ingredient would thereby be the first beneficiaries of a similar opportunity.

Therefore, the purposes of this study were (1) to evaluate the predictive ability of MIRS and NIRS for fine composition of individual BC, specifically EAA, and major and minor minerals, (2) to investigate the major nongenetic factors affecting the phenotypic variability of such traits, and finally (3) to assess correlations with the narrow-sense quality trait of BC, the IgG concentration (Costa et al., 2023).

MATERIALS AND METHODS

Experimental Design

Ethical approval was not required for the present study as per institutional guidelines or local legislation: only the owners had interactions with their animals and signed a written informed consent to be involved in the present study. Further details and a complete description of the experimental design can be retrieved in Costa et al. (2021). Briefly, 678 colostrum samples were collected from Holstein cows between 2019 and 2020 in commercial herds located in Northern Italy. Inclusion criteria for farmers ($n = 9$) was the lack of vaccination of cows before calving. This was intended to avoid presence of hyperimmune BC.

One BC sample per cow was available, covering parity from 1 to 8 and all calving seasons (Costa et al., 2021; Franzoi et al., 2022). The sampling procedure required calf to be immediately separated from the dam and the first colostrum to be collected within 6 h from parturition and immediately stored at -20°C . Approximately 120 mL of colostrum per cow was available for qualitative and quantitative analyses.

For the purpose of this study both reference data (AA and minerals) and spectra were needed. In some cases, samples did not have all the aliquots required for the determination of fine composition traits or, vice versa, for the acquisition of either the MIRS or the NIRS spectrum. This resulted in a loss of sample compared with the original number ($n = 678$; Costa et al., 2021).

Chemical Analyses

After thawing samples at 4°C , an aliquot was lyophilized for gross and fine composition determination (Goi et al., 2023). Fat, protein, and lactose contents of colostrum were determined as described in Goi et al. (2023) using the Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten VI C15.2.1 method (VDLUFA, 2013), the Kjeldahl method (AOAC International, 2000), and the HPLC, respectively. Chemical analyses were carried out at the LaChi laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Italy).

For minerals quantification, a 2-mL aliquot underwent mineralization with 7 mL of 67% nitric acid (HNO_3) and 2 mL of 30% hydrogen peroxide in closed vessels by microwave technique (200°C for 15–18 min, cooled to 35°C , and made up to volume with distilled water) using the Milestone Start D instrument (Milestone S.r.l. Sorisole, BG, Italy) equipped with Rotor SK-10 at high pressure and with a temperature probe.

This phase was followed by quantification with ICP-OES Spectro Arcos (Spectro Analytical Instruments GmbH, Kleve, Germany) at 315.887 (Ca), 177.495 (P), 182.034 (S), 766.941 (K), 589.592 (Na), 285.213 (Mg), 213.856 (Zn), and 259.941 nm (Fe) according to the method described by Poitevin (2016). A further aliquot in unprocessed (15 mL) form was lyophilized and then used for the quantification of AA which were analyzed after acid hydrolysis and pre-column derivatization, separated by reversed-phase HPLC and analyzed by UV detection. The method, adapted from European Pharmacopoeia (2003), included hydrolysis with hydrochloric acid (6 M) at 105°C for 24 h for Leu, Lys, Thr, Val, Phe, Arg, Ile, His, and Met. Subsequently, samples were neutralized with sodium hydroxide (8 M), the volume was adjusted to 100 mL with deionized water in a volumetric flask, and passed through a 0.45- μ m filter. The derivatization step of the mixture containing 10 μ L of a protein hydrolysate extract was conducted according to the manufacturer instructions (AccQ-Tag Ultra Derivatization Kit, Waters Corporation, Milford, MA). In the case of Trp, the concentration was determined following a method adapted from CD 2000/45/EC (European Union, 2000), by using a basic hydrolysis with barium hydroxide at 105°C for 24 h. Separation and quantification of the AA were performed using an Agilent 1260 Infinity HPLC (Agilent Technologies, Santa Clara, CA) equipped with a reversed-phase column C18 (Cortecs C18, 2.7 μ m, 2.1 $\times$ 150 mm; Waters Corporation, Milford, MA) kept at 45°C, and with a diode array Detector (Agilent 1260 Series, DAD VL+). The 10 EAA analyzed were selected following NRC (2001).

Infrared Spectra Collection

Each sample of BC was added to 25 mL of pure water, resulting in a 1:1 dilution ratio, prior MIRS spectra collection through the MilkoScan 7 RM (FOSS Electric A/S, Hillerød, Denmark) located in the milk laboratory of the Veneto Breeders' Association (Vicenza, Italy). The instrument, working in reflectance mode, was kept at room temperature (20°C) and operated in a wavelength range between 5,011.54 and 925.92 cm^{-1} every 3.85 cm^{-1} (1,060 wavelengths). The DS2500 (FOSS Electric A/S, Hillerød, Denmark), located in the La-Chi laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Italy), was adopted for NIRS spectra acquisition (25,000 to 4,000 cm^{-1} , absorbance) using 10 mL of undiluted BC. Spectra were extrapolated by mean of the FOSS Integrator software (FOSS Electric A/S, Hillerød, Denmark) to be coupled with the chemical reference values for the development of the prediction equation. Distribution of reference

data were visually inspected and kurtosis and symmetry were obtained through the PROC UNIVARIATE (SAS v.9.4; SAS Institute Inc.) to ensure that kurtosis and skewness fall between -1 and 1. In the case of Fe, whose data points presented a skewed distribution, values were normalized through a $\log_{10}$ transformation.

Chemometric Analysis

A self-built code based on partial least square (PLS) regression was used to train in loop and test the model of Leu, Lys, Thr, Val, Phe, Arg, Ile, His, Trp, Met, Ca, P, S, K, Na, Mg, Zn, and Fe. For the evaluation of prediction accuracy, both a k-fold cross-validation (calibration set, 75% of data) and an external validation (external validation set, 25% of data) were adopted. In particular, for each trait separately, the data set was split as follows: first, reference values were sorted in ascending order according to the concentration of the target trait, then a macro was used for sampling to extract every fourth sample for the validation set. Therefore, the remaining samples represented the calibration set and were used for model training. The number of samples present in the 2 sets differed according to the number of records available for a given trait. This selection ensured that both sets were representative of cows' parities, calving seasons, and herds. Moreover, as the database did not contain repeated samples of the same cow, none of the animals present in the validation set was used for model validation. For each trait, therefore, both the mean and the variability were similar in the 2 subsets.

Before the PLS, the spectrum of each individual sample underwent a standard normal variate scatter correction as pre-treatment to normalize the data and avoid possible deviations from the linear relationship between the spectrum and the reference value (Rinnan et al., 2009). Mid-infrared regions associated with water absorption and causing random noise (5,011 to 2,974 cm^{-1} , 2,503 to 1,929 cm^{-1} , 1,712 to 1,585 cm^{-1} , and 964 to 925 cm^{-1}) were eliminated. This practice is widely accepted in the case of analyses on bovine mammary secretions based on the investigations conducted by Hewavitharana and van Brakel (1997), Grelet et al. (2015), Visentin et al. (2016), and Frizzarin et al. (2021). Mahalanobis distance from the mean spectrum (Global H > 3) was used to identify and discard spectral outliers (Williams, 2007). An overview of final number of records available is given in Table 1, net of missing values and outliers deleted during the editing. For the reference traits, values that deviating more than 3 standard deviations from the mean were considered as missing, and samples whose spectra greatly deviated from the average spectrum were discarded.

In the calibration set, data were mean centered and scaled, then regression analysis was performed. Three steps of internal cross-validation were carried out to train the model for each trait using 5 random subsets, and every step involved each of the 5 groups once as a validation set, with the remaining 4 groups serving as calibration sets. After each iteration, the root mean square error of cross-validation (RMSE_{CV}) was calculated and an outlier elimination was performed when the predicted and the reference value differed more than 2.5 standard error of cross-validation. The number of latent variables (LV) included in the final model was the one that minimized the root mean square error and limited potential overfitting (Franzoi et al., 2022). For each trait, the variable importance in projection (VIP) scores of the last round of PLS were kept to identify the most relevant spectral regions for prediction. Subsequently, the best models developed were applied to the validation set to test the predictive performance on external samples. The performances of the final calibration model were the coefficient of determination of cross-validation (R^2_{CV}) and external validation (R^2_{V}) and the residual predictive deviation (RPD). As a dimensional, the RPD is commonly adopted in spectroscopy for standard evaluation of models' quality (Prevolnik et al., 2010) and can be considered thereby more objective than RMSE. The latter gives information about the overall difference between reference data and their predictions and is the criterion by which choosing the best model when several are tested for the same trait. SAS software v. 9.4 (SAS Institute Inc.) was used for data manipulation and editing and for model calibration and validation as well as for checking the normality of the resulting residuals. Finally, a t -test was performed to verify that the bias did not statistically differ from zero.

Analysis of Variance

To address the second objective of the present study, the phenotypic variability of reference data were analyzed through PROC GLM (SAS v.9.4; SAS Institute Inc.). The model can be described as follows:

$$y_{ijkl} = \mu + S_i + P_j + Y_k + H_l + (S \times P)_{ij} + e_{ijkl},$$

where y is the vector containing phenotypic records; μ is the intercept of the model; S_i is the fixed effect of the i th season of calving (December to February, March to May, June to August, and September to November); P_j is the fixed effect of the j th parity (3 classes, where the last included parity orders from 3 to 8); Y_k is the fixed effect of the k th year of calving (2019 and 2020); H_l is

the fixed effect of the l th herd; $(S \times P)_{ij}$ is the fixed interaction effect between season of calving and parity; and e_{ijkl} is the random residual assumed to be distributed as $\sim N(0, \sigma_e^2)$, where σ_e^2 is the residual variance. Differences between the least squares means (LSM) were tested using the Bonferroni multiple comparison post-hoc test ($P < 0.05$), and the PROC CORR (SAS v.9.4; SAS Institute Inc.) was used to calculate Pearson correlations and their significance.

Quality Classification

The narrow-sense quality of BC is essentially given by the IgG concentration (Godden, 2008) which, ideally, must be equal or greater than 50 g/L to ensure an adequate passive transfer of immunity. To visualize how samples with high and low IgG concentrations cluster according to the AA and minerals, 2 categories were created: low quality (IgG < 70 g/L, $n = 173$) and good quality (IgG ≥ 70 g/L, $n = 387$). The cut-off was set at 70 g/L due to the small number of samples ($n = 88$) with IgG below the conventional threshold (50 g/L; Godden, 2008), and 52 samples were excluded due to the absence of one or more predictors (i.e., missing value for any AA or minerals), leading to 159 and 349 samples for low quality and good quality, respectively. In SAS (SAS v.9.4; SAS Institute Inc.), PROC PRINCOMP was adopted for graphical visualization of samples through principal component (PC) scores, and PROC DISCRIM with cross-validation followed by backward elimination (PROC STEPDISC) was used to evaluate AA and mineral classification ability by mean of the sensitivity and the specificity.

RESULTS AND DISCUSSION

Relevance for Stakeholders

Milk of cows, buffalo, ewes, and goats is routinely analyzed via MIRS worldwide (ISO, 2013). This is not the case for colostrum, whose physical and chemical properties—such as density and Ig composition—make it a matrix very different from transition and mature milk. For these reasons, usually colostrum is not collected by dairies for milk and dairy products manufacturing, but is exclusively intended for feeding the young stock of the farm, and consequently it rarely undergoes analysis in certified laboratories. Spina et al. (2021) applied the prediction equations of bovine milk to colostrum samples and concluded that existing milk MIRS models may be used for an overall screening of colostrum composition in cattle. However, the portfolio of commercial milk models does not include important

Table 1. Descriptive statistics¹ of EAA (mg/100 g, as is) and mineral (mg/kg, as is) concentrations measured in bovine colostrum via gold standards and their Pearson correlations ($P < 0.05$) with Ig fractions and total Ig concentration

Trait	n	Mean	Range	CV, %	IgG	IgA	IgM	Total Ig
AA								
Leu	560	301.31	42.57–722.21	40.21	0.66	0.36	0.52	0.62
Lys	558	287.07	44.87–723.06	40.71	0.66	0.37	0.53	0.64
Thr	560	230.89	31.63–606.18	44.73	0.75	0.43	0.56	0.73
Val	546	192.92	25.55–524.83	42.47	0.71	0.38	0.55	0.69
Phe	560	142.62	23.91–345.20	39.35	0.66	0.37	0.53	0.63
Arg	560	132.80	16.35–339.44	43.85	0.71	0.42	0.56	0.69
Ile	559	106.18	14.62–264.60	39.47	0.61	0.34	0.51	0.58
His	560	99.38	6.73–302.98	41.36	0.65	0.38	0.51	0.62
Trp	560	70.84	12.34–207.33	45.39	0.75	0.46	0.57	0.75
Met	546	49.02	9.89–136.35	43.58	0.43	0.15	0.34	0.38
Mineral								
Ca	555	2,212.23	631.48–3,694.25	23.28	0.17	−0.02 ^{NS}	0.19	0.15
P	556	1,978.20	835.12–3,113.15	19.94	0.08 ^{NS}	−0.05 ^{NS}	0.13	0.08 ^{NS}
S	557	1,467.05	423.17–2,405.79	24.61	0.82	0.55	0.63	0.82
K	557	1,423.58	729.96–2,068.66	15.09	−0.22	−0.06 ^{NS}	−0.12	−0.16
Na	551	557.78	250.39–1,065.13	25.73	0.09	−0.07 ^{NS}	0.11	0.02 ^{NS}
Mg	552	344.51	116.07–637.18	26.33	0.41	0.18	0.35	0.37
Zn	555	20.30	4.99–41.19	34.83	0.26	0.04 ^{NS}	0.26	0.25
Fe ²	545	1.38	0.32–4.59	43.48	0.19	0.13	0.18	0.18

¹Before outlier elimination.²Log₁₀-transformed.

components of colostrum such as Ig and growth factor nor fine composition traits such as minerals, AA, and vitamins. Some authors attempted to develop infrared models for colostrum, but data size resulted in their case a limiting factor for further investigations (Navrátilová et al., 2006; Rivero et al., 2012; Elsohaby et al., 2018). Only recently, Franzoi et al. (2022) and Goi et al. (2023) developed colostrum-specific calibration models using Holstein samples and the accuracy obtained for IgG concentration and protein content was satisfactory and promising ($R^2_v \geq 0.83$). Satisfactory R^2_v have also been obtained in MIRS prediction of BC protein (0.89), fat (0.74), and lactose content (0.86; Goi et al., 2023). No studies evaluated the MIRS ability to predict colostrum AA and minerals. Reasonably, MIRS and NIRS models that are accurate enough have the potential to attract stakeholders of the food, feed, and pharmaceutical industry. In this study thawed BC was used to mimic the most common situation: the BC in the farm is most of the time frozen, representing the internal colostrum bank.

Starting Data

The number of samples originally available for each trait is reported in Table 1 together with the BC average composition. Table 2 presents the descriptive statistics of the 2 sets, the one adopted for calibration plus cross-validation and the one used for external validation, where the number of observations differed for each parameter according to the data available be-

fore and after editing. For all the investigated AA and minerals, including the logarithmically transformed Fe, data points were normally distributed. Within the AA, the greatest concentration on a fresh sample basis was found for Leu (301.31 mg/100 g) and Lys (287.07 mg/100 g). The Met was instead scarcely present with a concentration averaging 49.02 mg/100 g. These outcomes are in line with the order observed by Rafiq et al. (2016) in bovine milk, who found a high content of Leu followed by Lys, and a low content of Met and His. The coefficients of variation (CV) of AA were similar, ranging from a minimum of 39.4% (Phe) to a maximum of 45.4% (Trp). All the correlations with the Ig fractions were significant and positive, with an average magnitude of 0.54 (Table 1); in particular, the weakest association was observed between Met and IgA, and the strongest was between Trp and total Ig. Overall, the top minerals in terms of concentration were Ca and P (2,212.23 mg/100 g and 1,978.20 mg/100 g, respectively), which showed a CV around 20% (Table 1). Among the micro-minerals quantified, instead, Zn (20.30 mg/100 g) was the most abundant. Considering all minerals, the CV ranged from 15.1% to 43.5% for K and Fe, respectively. Some correlations with the colostrum Ig fractions were not significant, particularly in the case of IgA (Table 1). Nevertheless, S was strongly correlated with all fractions, and only K presented significant negative coefficients (Table 1; e.g., with IgG, −0.22). For chemical reasons, AA can reasonably be associated with protein compounds, even in BC. Therefore, the average CV of the 10 AA investigated in this

Table 2. Overview of descriptive statistics of the traits¹ determined via gold standards in the calibration set (75% of data) and in the external validation (25% of data) set

Trait	Set	n	Mean	Range	CV, %
AA					
Leu	Calibration	420	300.64	42.57–702.58	40.13
	Validation	140	303.33	67.33–722.21	40.58
Lys	Calibration	419	286.94	44.87–723.06	40.84
	Validation	139	287.44	63.66–713.52	40.47
Thr	Calibration	420	230.37	31.63–590.68	44.66
	Validation	140	232.43	43.55–606.18	45.09
Val	Calibration	410	192.94	25.55–524.83	42.76
	Validation	136	192.86	39.31–455.96	41.75
Phe	Calibration	420	142.32	23.91–327.94	39.28
	Validation	140	143.39	34.40–345.20	39.70
Arg	Calibration	420	132.51	16.35–336.38	43.78
	Validation	140	133.66	24.61–339.44	44.19
Ile	Calibration	420	106.25	14.62–264.60	39.77
	Validation	139	105.98	25.10–247.60	38.68
His	Calibration	420	99.10	6.73–289.93	41.01
	Validation	140	100.24	22.73–302.98	42.50
Trp	Calibration	420	70.65	12.34–192.50	45.14
	Validation	140	71.41	14.13–207.33	46.25
Met	Calibration	410	49.03	9.89–136.35	43.86
	Validation	136	48.99	12.64–120.11	42.89
Mineral					
Ca	Calibration	417	2,212.49	631.48–3,694.25	23.45
	Validation	138	2,211.45	944.25–3,586.02	22.85
P	Calibration	417	1976.15	835.12–3,091.73	19.96
	Validation	139	1984.35	1,010.76–3,113.15	19.93
S	Calibration	418	1,465.97	423.17–2,405.79	24.69
	Validation	139	1,470.28	545.25–2,361.70	24.47
K	Calibration	418	1,422.73	729.96–2,068.66	15.13
	Validation	139	1,426.15	805.66–2,051.34	15.03
Na	Calibration	414	557.95	250.39–1,065.13	25.92
	Validation	137	557.26	312.78–1,020.32	25.24
Mg	Calibration	414	344.02	116.07–613.09	26.31
	Validation	138	345.99	169.07–637.18	26.49
Zn	Calibration	417	20.30	4.99–41.19	35.03
	Validation	138	20.29	6.72–39.79	34.36
Fe ²	Calibration	409	1.38	0.32–4.59	43.40
	Validation	136	1.38	0.56–4.21	43.25

¹Expressed as mg/100 g, as is, for AA and milligrams per kg, as is, for minerals.²Log₁₀-transformed.

study (42.11%, data not shown) is expected to be similar to the CV of Ig, especially IgG (39.56%; Costa et al., 2021). In contrast, minerals exhibited less variation than AA (Table 1), being the CV on average 26.66%. This is likely due to the nature of the traits, as minerals can vary independently of Ig concentration and may be associated with other—even nonorganic—compounds. In addition, the biological mechanisms responsible for the transfer of minerals from the dam's blood to her mammary gland secretion are different from those involved in the transfer of Ig.

It is rather difficult to compare results given in Table 1 with the literature, due to paucity of published data on the fine composition of BC. There are small-case studies where the average and the range of minerals content differ from this study mainly due to the sampling procedures (e.g., collection time) and smaller sample size (Tsioulpas et al., 2007; Abd El Fattah et

al., 2012; Playford and Weiser, 2021). In the paper of Tsioulpas et al. (2007), the 8 colostrum samples collected the day after calving presented lower Ca and P compared with this study, likely due to the dramatic change in mineral content of the mammary gland secretion in the first hours postpartum (Sodhi et al., 1996; Godden, 2008; Costa et al., 2021). Abd El-Fattah et al. (2012) found comparable levels of Ca, Mg, and K in the colostrum of 12 calving cows, but a significantly higher Na concentration approximately 4 times that of the current study, and approximately a quarter of the P content. Playford and Weiser (2021) showed some intervals of mineral content values in colostrum, and the findings from the present study align closely with the lower end of that range, except for P, which falls significantly below the indicated ranges. The high variability of cow's colostrum composition has been widely discussed in the literature (Playford and Weiser, 2021)

and might explain the abovementioned differences. As an example, Kehoe et al. (2007) conducted a study involving 55 freeze-dried BC samples collected in the United States, and the average contents of Ca, P, K, and S were 4,716.10, 4,452.10, 2,845.89, and 2,595.67 mg/kg respectively, which are roughly double the values observed in the present research. Furthermore, their study exhibited a remarkable level of variability in these mineral concentrations. However, it was not possible to understand if the concentrations were on a fresh or DM basis. Finally, it is important to consider that some dissimilarities with previous studies can be partly attributed to farming system and characteristics of animals involved (breed, parity; Costa et al., 2021) other than to the wet laboratory methodology used for quantification. Such evident divergences make a clear and fair comparison not always possible.

Predictive Ability of MIRS and NIRS

In the field, rapid tools for evaluation of the colostrum Ig concentration such as refractometers are getting popular. The Ig concentration is, in fact, the unique colostrum intrinsic quality trait that can be monitored nowadays in well managed farms. The refractive index (°Brix) of digital and analogic refractometers provides an indication of total solids content of a colostrum sample, which is indirectly correlated with proteins and Ig (Costa et al., 2022). However, as the Ig represent the 80% of total protein content, it is difficult to approximate AA and even more mineral concentrations using these devices. Unfortunately, even though important for the newborn and for characterization of the total quality of this matrix, substances such as lactoferrin, peptides, fatty acids, AA, and minerals are present in traces compared with the Ig (Gomes et al., 2021), making the use of the refractive index not appropriate for their assessment. For this reason, for fine composition traits there is the opportunity to explore the predictive ability of MIRS, a technology well known by the dairy community, and of NIRS. External validation was performed on a pool of samples with characteristics and variability similar to the calibration set (Table 2). Prediction performances of the best models in cross- and external validation are summarized in Table 3 and Table 4. As regards to the AA predicted via MIRS, the outliers ranged between 2.93% and 6.43%, and the number of LV included in the final PLS round varied from 5 to 15. Overall, R^2_{CV} were satisfactory, being in the range between 0.67 (Met) and 0.86 (Arg, His, and Thr); Val obtained the best accuracy in external validation ($R^2_V = 0.81$; $RPD = 2.28$), followed by Thr and Leu. Values of RPD greater than 2 indicate that the predictions must be merely intended for screening and

not for punctual determination (Williams, 2014). The prediction models for Lys, Phe, Arg, Trp, Leu, Thr, and Val obtained R^2_V above 0.66 suggesting that MIRS can provide an approximated estimate of their content (Williams, 2003). In external validation, Ile, Met, and His were not predicted with enough precision to use the MIRS for a satisfactory quantification. McDermott et al. (2016) predicted Lys, Val, and Arg content of bovine milk using MIRS but reached a lower accuracy in both cross- and external validation (e.g., with coefficients of correlation of 0.55, 0.59, and 0.26, respectively). When NIRS was applied, the percentage of outliers detected felt within the range previously identified with MIRS. Results for R^2_{CV} were as good as those using MIRS, ranging from 0.76 (Met) to 0.87 (Thr and Phe), whereas the best performance in external validation was reached for Phe ($R^2_V > 0.82$; $RPD > 2.5$), indicating the possibility of a fair screening for this specific trait. Overall, NIRS required a greater minimum number of LV (10), and its prediction ability was satisfactory, showing the capability to roughly screen for almost all the 10 AA.

The accuracy of colostrum-minerals prediction models is presented in Table 4. For prediction by MIRS, the outliers detected in calibration were on average 5%, and the number of LV ranged from 4 to 15. Colostrum S reached an excellent accuracy in cross-validation ($R^2_{CV} = 0.94$), followed by P and Ca whose R^2_{CV} was greater than 0.82 indicating a good prediction of the reference values. The prediction generated for Mg was good for approximately quantifying the mineral, whereas the Na and Zn models can be considered at most able to discriminate between lowly and highly concentrated samples (Williams, 2003). Finally, statistics obtained for the other minerals (i.e., K, Zn, and Fe) were not sufficiently high to consider the practical use of their prediction equation, and this could be attributed to the low concentration in which at least Zn and Fe are present. Further investigation will be needed to explore the reasons for the unsatisfactory performance of MIRS for Zn prediction. The external validation performed on the 25% of total samples available (out of the calibration set) produced models with an ability that can provide approximate predictions exclusively for P, S, Mg, and Ca. The R^2_V , in fact, varied between 0.66 and 0.80. Especially for S, the $RMSE_V$ was quite far from the $RMSE_{CV}$, indicating a certain loss in prediction accuracy and instability of the model when dealing with external or independent samples. The RPD mirrors the differences in prediction models' reliability and, according to the interpretation of Williams (2014), only the model developed for P can be applicable for a rough screening, whereas others would not be recommended. In milk, Visentin et al. (2016) achieved lower MIRS accuracy in cross-validation for Ca, Mg, Na, and P but

Table 3. Fitting statistics¹ of modified partial least square regression analysis in 5-fold cross-validation and external validation for bovine colostrum EAA (mg/100 g, as is)

Trait ²	n	Out	RMSE _{CV}	R ² _{CV}	n _{ext}	LV	RMSE _V	R ² _V	RPD
MIRS									
Leu	397	5.48	43.95	0.85	140	14	60.55	0.76	2.03
Lys	399	4.77	46.62	0.82	139	15	63.74	0.70	1.83
Thr	396	5.71	35.44	0.86	140	13	49.29	0.78	2.13
Val	392	4.39	32.26	0.82	136	15	35.29	0.81	2.28
Phe	400	4.76	20.35	0.85	140	14	28.95	0.74	1.97
Arg	396	5.71	20.34	0.86	140	12	29.94	0.74	1.97
Ile	401	4.52	18.27	0.78	139	12	24.79	0.64	1.65
His	395	5.95	14.36	0.86	140	5	30.72	0.48	1.39
Trp	393	6.43	12.69	0.80	140	11	16.73	0.75	1.97
Met	398	2.93	11.36	0.67	136	9	13.40	0.60	1.57
NIRS									
Leu	455	4.41	42.41	0.86	158	14	61.24	0.73	1.93
Lys	452	4.64	44.73	0.84	158	15	48.29	0.83	2.39
Thr	458	3.78	35.45	0.87	158	10	46.48	0.79	2.16
Val	443	4.73	28.54	0.86	155	13	38.67	0.77	2.09
Phe	453	4.83	19.46	0.87	158	15	20.62	0.86	2.67
Arg	456	4.00	20.35	0.86	158	14	27.28	0.77	2.07
Ile	451	4.85	16.81	0.82	158	11	22.35	0.71	1.85
His	452	4.84	14.32	0.86	158	13	18.84	0.77	2.08
Trp	448	5.49	12.29	0.83	158	15	16.36	0.72	1.89
Met	443	4.73	9.11	0.76	155	11	12.13	0.65	1.69

¹n = number of samples used to perform the calibration after outlier elimination; Out = percentage of outliers in the calibration set; LV = latent variables; RMSE_{CV} = root mean square error of cross-validation; R²_{CV} = coefficient of determination of cross-validation; n_{ext} = number of samples used to perform the external validation; R²_V = coefficient of determination of external validation; RPD = residual predictive deviation.

²MIRS = mid-infrared spectroscopy; NIRS = near-infrared spectroscopy.

a better R²_{CV} for K (0.69). Still in bovine milk, Soyeyurt et al. (2009) obtained a greater but still unsatisfactory R²_{CV} for Na (0.65), similar results for Ca (0.87) and P (0.85), and lower for K (0.36) and Mg (0.65). In

contrast, among samples available for NIRS prediction, the number of outliers averaged 4%, and the range of LV used for the last PLS was slightly greater than that of MIRS, including lower values. Only S reached a suf-

Table 4. Fitting statistics¹ of modified partial least square regression analysis in 5-fold cross-validation and external validation for bovine colostrum minerals (mg/kg, as is)

Trait ²	n	Out	RMSE _{CV}	R ² _{CV}	n _{ext}	LV	RMSE _V	R ² _V	RPD
MIRS									
Ca	403	3.36	193.55	0.86	138	9	297.38	0.66	1.70
P	392	6.00	130.63	0.88	139	11	178.20	0.80	2.22
S	393	5.98	86.02	0.94	139	6	183.53	0.74	1.97
K	403	3.62	146.20	0.46	139	8	175.09	0.34	1.22
Na	406	1.93	94.07	0.54	137	15	96.38	0.53	1.49
Mg	395	4.59	38.94	0.79	138	12	52.44	0.68	1.73
Zn	400	4.08	4.03	0.62	138	12	4.97	0.50	1.40
Fe ³	374	8.56	0.27	0.40	136	4	0.54	0.20	1.11
NIRS									
Ca	464	1.90	341.08	0.52	157	11	362.97	0.49	1.39
P	466	1.69	258.95	0.53	158	13	280.49	0.52	1.44
S	459	3.77	101.09	0.92	158	15	133.14	0.87	2.78
K	460	2.95	185.19	0.04	158	2	207.48	0.05	1.02
Na	442	6.16	109.38	0.05	157	3	141.53	0.08	1.04
Mg	454	4.02	52.87	0.63	157	8	66.99	0.47	1.37
Zn	461	2.74	5.13	0.48	158	7	5.51	0.45	1.35
Fe	434	7.07	0.25	0.71	155	8	0.56	0.24	1.14

¹n = number of samples used to perform the calibration after outlier elimination; Out = percentage of outliers in the calibration set; LV = latent variables; RMSE_{CV} = root mean square error of cross-validation; R²_{CV} = coefficient of determination of cross-validation; n_{ext} = number of samples used to perform the external validation; R²_V = coefficient of determination of external validation; RPD = residual predictive deviation.

²MIRS = mid-infrared spectroscopy; NIRS = near-infrared spectroscopy.

³Log₁₀-transformed.

ficient accuracy to consider the possibility of using the model for approximate quantification of S content ($R^2_v = 0.87$; RPD = 2.78). Such difference in mineral prediction accuracy when changing the technology used to collect spectra could be explained by a different mode of operation. Indeed, the NIRS instrument works in reflectance mode, and the viscosity of the matrix might affect the diffuse reflectance of light (Williams, 2007). In fact, NIRS is more sensitive to the inhomogeneity of the material compared with the transmission mode of MIRS, because the former technology analyzes only the surface with diffused reflectance, whereas the latter scans a larger volume of sample (Ito et al., 2008).

The most important spectral regions for AA and minerals prediction were identified through the VIP scores (Figure 1; Figure 2). In particular, wavenumbers common to all AA or minerals with a VIP score greater than 1 (Caponigro et al., 2023) were considered as the most significant. Overall, regardless of the instrument, the wavenumbers with VIP >1 for minerals fall within the most important ranges of AA (Figure 1; Figure 2). In the mid-infrared region, the most informative spectral windows are known to be associated with protein absorption bands. Supporting this, Soyeurt et al. (2012) reported regions between 1,700 and 1,500 cm^{-1} and from 1,450 to 1,200 cm^{-1} to be associated with milk proteins; however, the absorption zone of milk carbohydrates, including lactose (1,250 to 900 cm^{-1}), is also covered by the identified wavenumbers (Caponigro et al., 2023). Among the most important spectral wavelengths, also in the near-infrared spectrum, there are proteins and AA absorption bands which could explain the good results obtained in the prediction. In detail, 5,051, 4,878, 4,690, and 4,359 cm^{-1} are wavenumbers that can be related to the N–H bond absorption of proteins and the N–H and C=O stretching absorption band of milk AA (De Benedictis and Huck, 2016). In addition, signals present between 4,630 and 4,651 cm^{-1} can be attributed to the combination band of amide I and amide III, whereas 4,425 cm^{-1} is linked to the N–H stretching and NH_3 deformation of AA and 4,329 cm^{-1} to N–H stretching and C–H deformation of AA. Lastly, signals around 6,798 and 6,849 cm^{-1} are dominated by the N–H stretching of amides (De Benedictis and Huck, 2016).

Correlations

For the first time, in this study correlations among the reference data of colostrum minerals, AA, and Ig fractions were calculated (Table 1; Figure 3). All Ig fractions were positively correlated with the 10 EAA (Table 1), with coefficients averaging 0.54 and reaching magnitude up to 0.75 (i.e., for Trp vs. IgG, Thr vs. IgG, and Trp vs. total Ig). In regards to minerals, some

of the correlations calculated with the Ig fractions were either not significant or negative (Table 1). Only S, Mg, and Fe were significantly and positively correlated with IgG, IgA, IgM, and total Ig (Table 1). The major fraction, IgG, was negatively correlated with K (−0.22) and presented positive weak correlations with other minerals. The great majority of IgA correlations were not significant.

In general, correlations between the 10 AA were very strong and positive. The weakest association was found between Met and Trp (0.60) and the strongest between Leu and Phe (0.99). The correlation between the minerals were more variable and sometimes also negative (Figure 3). Colostrum K was, in fact, negatively associated with both Na (−0.32) and S (−0.16), and the 2 most-correlated minerals were Ca and P (0.91). In general, Na and K were the minerals with the weakest associations. The correlations between the 2 sets of traits were rather weak, with S being the unique mineral with a coefficient of correlation with the AA greater than 0.60. In fact, leaving aside the fact that AA represent the building blocks of peptides and proteins, certain AA such as Met contain non-negligible amounts of S (Brosnan and Brosnan, 2006).

Analysis of Variance

The LSM estimated within this study (Table 5; Table 6) are referred to the first colostrum samples, as the sampling protocol allowed the collection exclusively within 6 h from calving and did not permit suckling (Costa et al., 2021). Several studies dealing with variability of colostrum Ig and gross composition did not use similar restrictions, resulting in highly variable traits with difficult to-be-interpreted patterns (McGee and Earley, 2019). In fact, colostrum composition and density vary substantially in the first hours after parturition and tend to rapidly change, approaching values of transition milk. To our knowledge, this is the first time a large data set is used to estimate LSM for the effect of parity and calving season for AA and minerals concentration of BC.

Apart from a few exceptions, the fixed effects included in the model were notable in explaining the variability of the investigated traits. Given the short period considered (2019 and 2020), the effect of year in this study resulted not significant in explaining any traits' variability. Furthermore, across all seasons, AA and mineral composition of colostrum from cows of different parity order were similar without significant differences.

The AA generally presented an unclear pattern across parities, with the highest LSM in the 2 extreme classes (Table 5). As an example, the LSM of the most

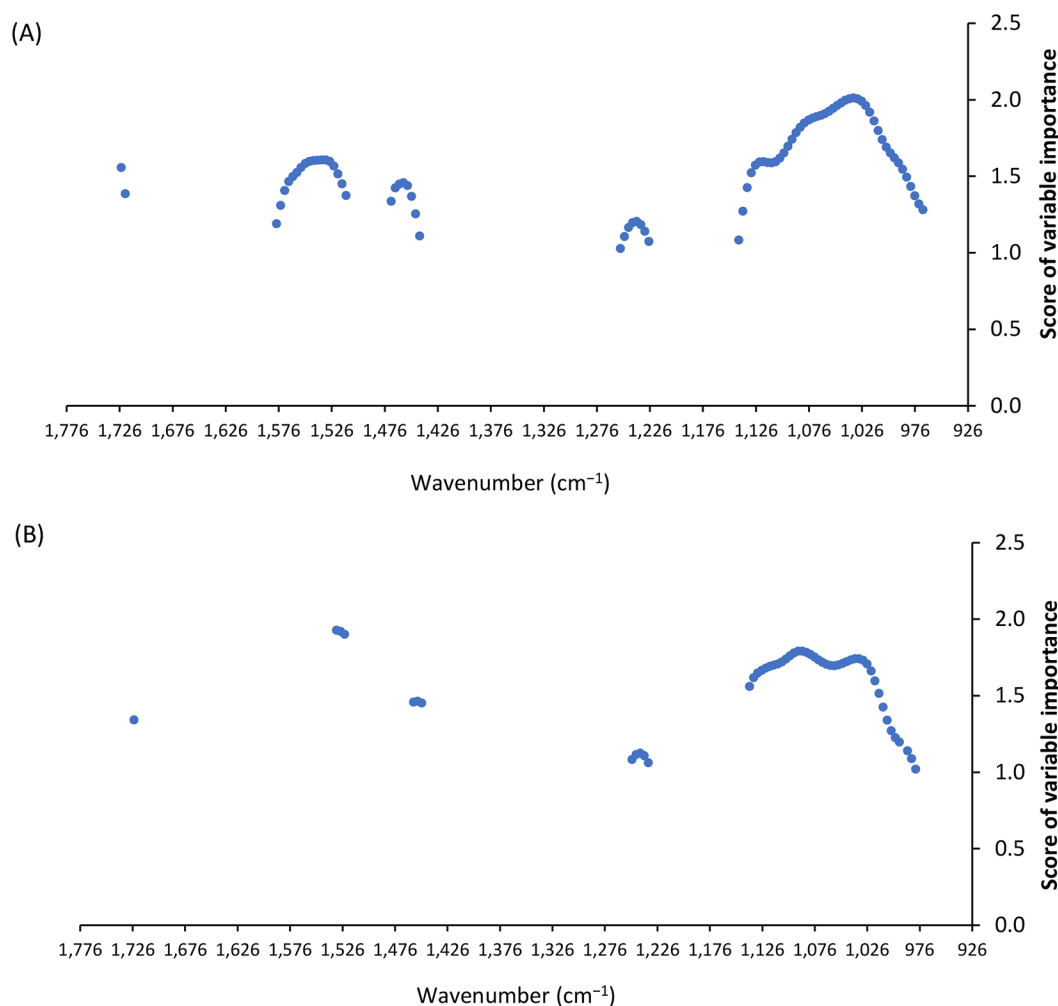


Figure 1. Overview of the most important (variable importance >1; Caponigro et al., 2023) wavenumbers (blue dots) used for prediction of bovine colostrum (A) EAA and (B) mineral concentration via mid-infrared spectroscopy.

abundant AA (Leu) were equal to 319.22, 261.41, and 335.75 mg/100 g for parity 1, 2, and ≥ 3 , respectively. Some major minerals, namely Ca, P, Mg, and Na, presented the greatest concentration in parity 1 (Table 6). The same can be said for Zn, whereas in the case of Fe, LSM estimated for parity 1 (1.35 ± 0.06 mg/100 g) and 3 (1.29 ± 0.05 mg/100 g) were similar and significantly different than the intermediate parity order (1.24 ± 0.06 mg/100 g). In contrast, S exhibited the largest LSM in older cows ($1,590.13 \pm 29.13$ mg/100 g). Valldecabres and Silva-del-Río (2022) confirmed a great amount of K in colostrum of second-parity cows compared to animals in parity ≥ 5 . The same authors found a pattern of P and Fe different than the ones of the present study. Visentin et al. (2018) investigated the phenotypic variability of infrared-predicted mineral concentrations of milk and estimated the greatest LSM of Ca, P, K, and Mg for milk produced by primiparous

cows with a progressive decrease along the productive life. Oppositely, the milk Na concentration increased with parity (Visentin et al., 2018). The effect of parity has been widely investigated on the BC Ig concentration, as it is the key parameter that determines the quality at farm level; there is consensus on the inferiority of younger cows compared with those that experienced more lactations (Bielmann et al., 2010; Costa et al., 2021). Bielmann et al. (2010) reported as better colostrum quality (i.e., with IgG equal or greater than the critical cut-off; 50 g/L), is usually found in multiparous cows. It is well known, also, that the colostrum density is lower in heifers ($1,059$ kg/m³) compared with multiparous cows ($1,068$ kg/m³; Strekozov et al., 2008). The mechanisms responsible for the transfer of nutrients from the dam blood to the colostrum are far from being considered completely understood in cattle. What is true is that some components vary, regardless of the

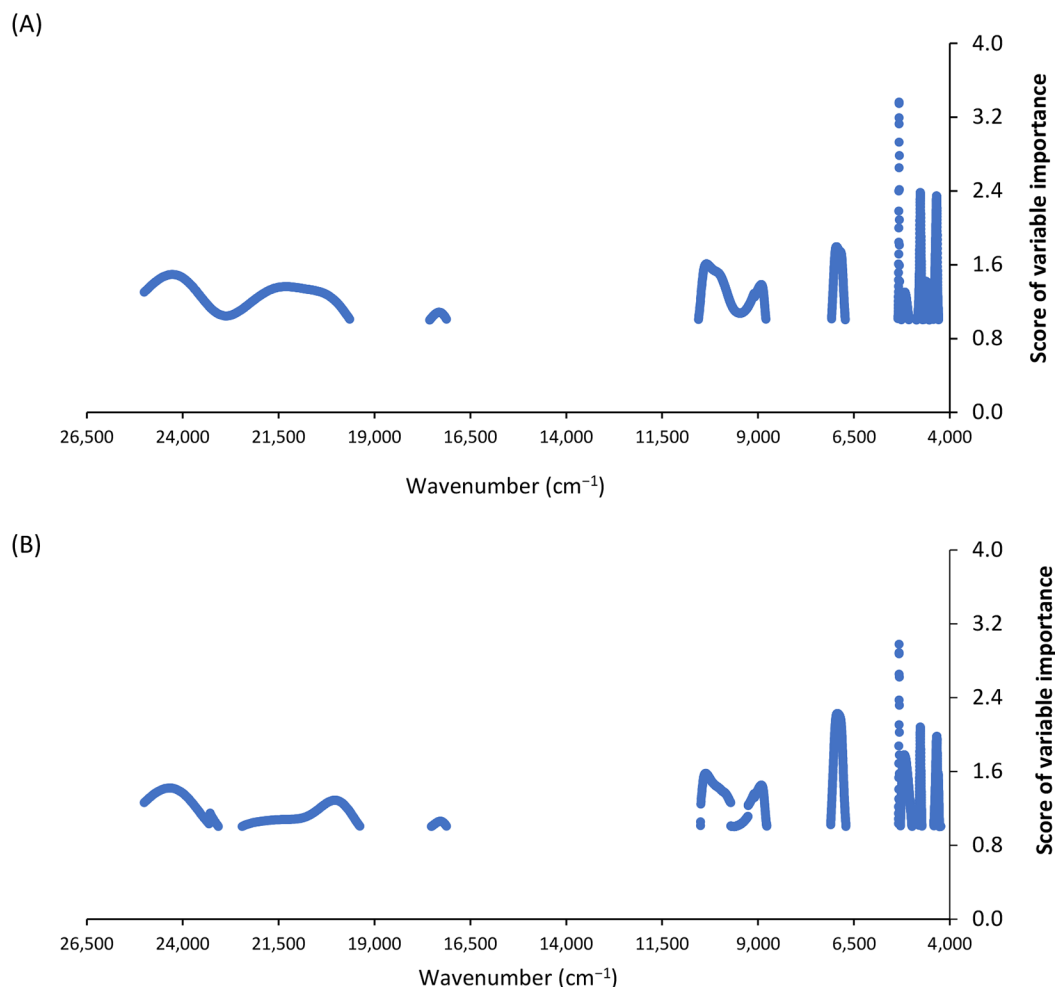


Figure 2. Blue dots represent the most important wavenumbers (variable importance >1; Caponigro et al., 2023) used for prediction of bovine colostrum (A) EAA and (B) mineral concentration via near-infrared spectroscopy.

Ig level (i.e., they are independent from the number of antibodies released in colostrum; Playford et al., 2020). As observed in milk, some AA and minerals are expected to fluctuate according to the diet administered to the cow (before or on the day of calving), whereas Ig concentration of colostrum cannot be dramatically and directly influenced by the feed composition and presence of supplements.

In regards to calving season, only Trp among the AA and all minerals except for Fe and Zn were affected by this effect. Colostrum Trp showed an LSM significantly lower in spring than autumn, whereas minerals highlighted an irregular arrangement; however, LSM of Ca indicated a significantly greater concentration in autumn and spring compared with summer, Zn was the highest in spring and summer and the lowest in winter. Supporting these findings, Miciński et al. (2017) showed the colostrum mineral composition to be af-

ected by calving season. However, their results are not comparable with those of present study because seasons classes were created by grouping the month differently. For the reasons explained above, the effect of herd which includes management and feed composition tended to be always significant except for Ca, Zn, and Fe. For traits present in small concentration compared with Ig and whose release in the alveoli depends on environmental rather than intrinsic animal factors such as feed composition and udder health (e.g., some minerals), the volume of colostrum yielded (**CY**, kg) by the cow represents an important factor. A sort of dilution of components, in fact, can be present in high-producing cows, while the opposite could be expected in animals yielding less. The CY at the first milking after calving can be largely variable: the 9 farmers involved in the study of Cabral et al. (2016) recorded values from 0.5 to 39.7 kg (average of 7.87 kg) and there was non-

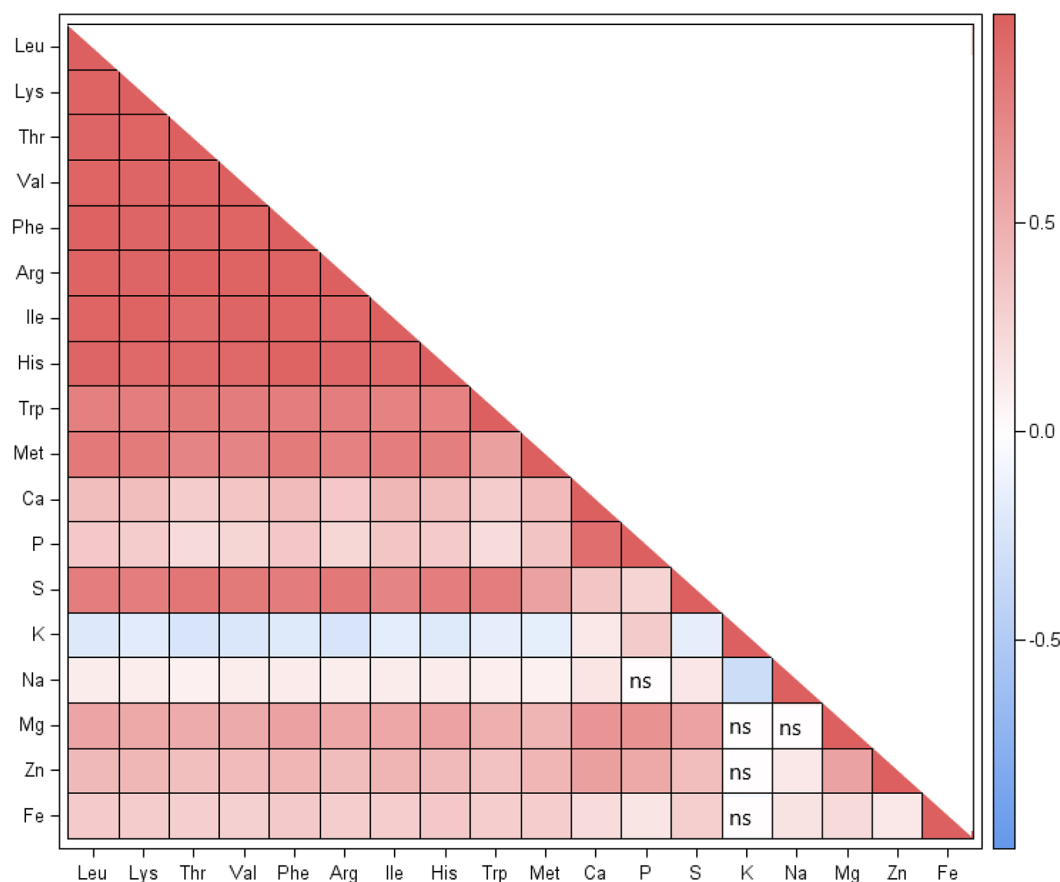


Figure 3. Heatmap with Pearson correlations ($P < 0.001$) between EAA and minerals of colostrum. Not significant coefficients are indicated with “ns.”

negligible variability across farmers. From the results of Strekozov et al. (2008) and considerations of Conneely et al. (2013), it can be hypothesized that the content of AA and minerals tends to be greater in periods where CY is lower (i.e., in autumn and winter in the northern hemisphere). Particularly in the case of minerals, what

is found in the mammary gland secretion reflects the blood levels. In addition, the high LSM observed in parity 1 and 3 for some AA (Table 5) can have 2 different explanations. In primiparous the high content of certain compounds could be due to a concentration effect (i.e., to their lower CY compared with pluriparous). More-

Table 5. Least squares means of colostrum essential AA (mg/100 g, as is) determined via gold standard in cross-validation for parity and calving season

Trait	Parity			Calving season			
	1	2	3 to 8	Spring	Summer	Autumn	Winter
Leu	319.22 ^a	261.41 ^b	335.75 ^a	290.49 ^a	295.96 ^a	310.29 ^a	325.11 ^a
Lys	293.27 ^a	243.43 ^b	312.74 ^a	272.39 ^a	283.88 ^a	288.16 ^a	288.15 ^a
Thr	232.98 ^b	195.47 ^c	262.65 ^a	213.54 ^a	229.75 ^a	238.87 ^a	239.30 ^a
Val	200.89 ^a	164.62 ^b	212.61 ^a	178.46 ^a	195.90 ^a	201.26 ^a	195.20 ^a
Phe	151.32 ^a	123.77 ^b	156.71 ^a	136.13 ^a	140.12 ^a	148.54 ^a	150.95 ^a
Arg	135.81 ^b	113.09 ^c	149.93 ^a	122.68 ^a	131.48 ^a	138.59 ^a	139.01 ^a
Ile	113.41 ^a	92.95 ^b	115.30 ^a	102.36 ^a	108.36 ^a	110.33 ^a	107.83 ^a
His	106.48 ^a	86.56 ^b	110.47 ^a	95.53 ^a	97.66 ^a	104.97 ^a	106.52 ^a
Trp	67.51 ^b	56.28 ^c	77.17 ^a	61.57 ^b	67.59 ^{ab}	73.81 ^a	64.98 ^{ab}
Met	52.00 ^a	41.55 ^b	50.16 ^a	50.26 ^a	47.65 ^a	38.51 ^b	55.20 ^a

^{a-c}Different letters within row and effect indicate significantly different estimates ($P < 0.05$).

Table 6. Least squares means of colostrum minerals (mg/100 g, as is) determined via gold standard in cross-validation for parity and calving season

Trait	Parity			Calving season			
	1	2	3 to 8	Spring	Summer	Autumn	Winter
Ca	2,428.40 ^a	2,077.04 ^b	2,121.49 ^b	2,276.22 ^a	2,076.72 ^b	2,250.35 ^a	2,232.61 ^{ab}
P	2,163.80 ^a	1,945.57 ^b	1,901.71 ^b	2,033.55 ^a	1,871.16 ^b	2,025.86 ^a	2,084.22 ^a
S	1,396.17 ^b	1,350.46 ^b	1,590.13 ^a	1,391.24 ^b	1,409.96 ^{ab}	1,508.74 ^a	1,472.41 ^{ab}
K	1,420.09 ^b	1,498.82 ^a	1,408.15 ^b	1,485.33 ^a	1,431.33 ^a	1,437.27 ^a	1,415.48 ^a
Mg	366.45 ^a	322.93 ^b	337.98 ^b	324.96 ^b	321.03 ^b	359.75 ^a	364.07 ^a
Na	599.36 ^a	526.28 ^b	561.56 ^b	586.20 ^a	580.57 ^a	573.55 ^a	509.29 ^b
Zn	23.08 ^a	18.88 ^b	19.09 ^b	21.26 ^a	21.51 ^a	19.92 ^{ab}	18.71 ^b
Fe ¹	1.43 ^a	1.24 ^b	1.42 ^a	1.35 ^a	1.31 ^a	1.38 ^a	1.41 ^a

^{a,b}Different letters within row and effect indicate significantly different estimates ($P < 0.05$).

¹Log₁₀-transformed.

over, cows in parity ≥ 3 might actually present a pronounced release or secretion of some substances in the colostrum produced. Nevertheless, in this experiment the individual CY was not recorded, making the validation of such hypotheses not possible. By using data of mature milk, which can differ from colostrum, some authors calculated correlations between milk yield and milk minerals concentration: results of Fadlalla et al. (2020) suggest that the relationship could be not linear. Milk Ca resulted to be positively associated with milk yield (0.36), whereas the correlation between milk yield and Na was weak and negative (−0.18). Finally, P, Mg and K presented correlation coefficients with milk yield close to zero. Ideally, to support our idea, CY and the amount of minerals yielded by the cow (mg/L) would be needed.

Detailed Composition and Ig Level

The output of the PC analysis is reported in Figure 4 and Figure 5. Principal component 1 explained 61.49% of the total variance, and PC 2 and PC 3 accounted for 12.83% and 6.99% of the total variation, respectively. The relationship between the BC quality level and fine composition (AA and minerals) based on the first 2 PC is graphically presented in Figure 4. The analysis suggested that most of the differences are given by the AA composition (PC 1 in Figure 4). In fact, when the PC 1 was plotted against PC 2 and PC 3 (Figure 5), it becomes clear that the 2 quality classes only partly overlapped and that a rough distinction is only possible based on PC 1 rather than other components (Figure 5). It derives that no clear separation can be observed when PC 2 is plotted against PC 3 (data not shown). Overall, this unsupervised approach suggested that AA might in part allow for an approximate distinction between samples with high and low IgG.

As regards the discriminant analysis, the final discriminant function included 4 traits, namely Val, Phe, Met, and S, and allowed for the correct classification of 77.7% ($n = 289$) of the good-quality samples and 81.55% ($n = 137$) of the low-quality samples. Similar performances were observed in cross-validation, with more than 3 quarters of samples correctly classified: 76.8% and 80.95% for good-quality and low-quality, respectively. Findings of both multivariate analyses reveal that the discriminant ability of AA is superior than that of minerals. Corroborating the correlations between AA and IgG discussed previously, these findings were in line with expectations. The AA are in fact precursors of peptides and proteins, including Ig (Chiu et al., 2019).

CONCLUSIONS

Results demonstrate how MIRS and NIRS can be used to predict BC detailed composition, including the concentration of 10 EAA. Satisfactory performance was achieved for Ca, P, S, and Mg in the case of MIRS ($R^2_V \geq 0.66$) and for S ($R^2_V = 0.87$) in the case of NIRS. Detailed BC composition is affected by both cow's parity and calving season. Although preliminary, findings suggest that some fine composition traits could be affected by dilution. Thus, future studies should account also for the amount of colostrum yielded by the cow to confirm this hypothesis. In the era of functional foods, rapid and low-cost technologies for the determination of BC fine composition are attractive for the food and pharmaceutical industries. Although improvable, having considered the promising prediction performance, we expect benchtop and pocket spectrometers to be an opportunity for farmers, animal scientists, and veterinarians for phenotyping and in-field BC quality evaluation.

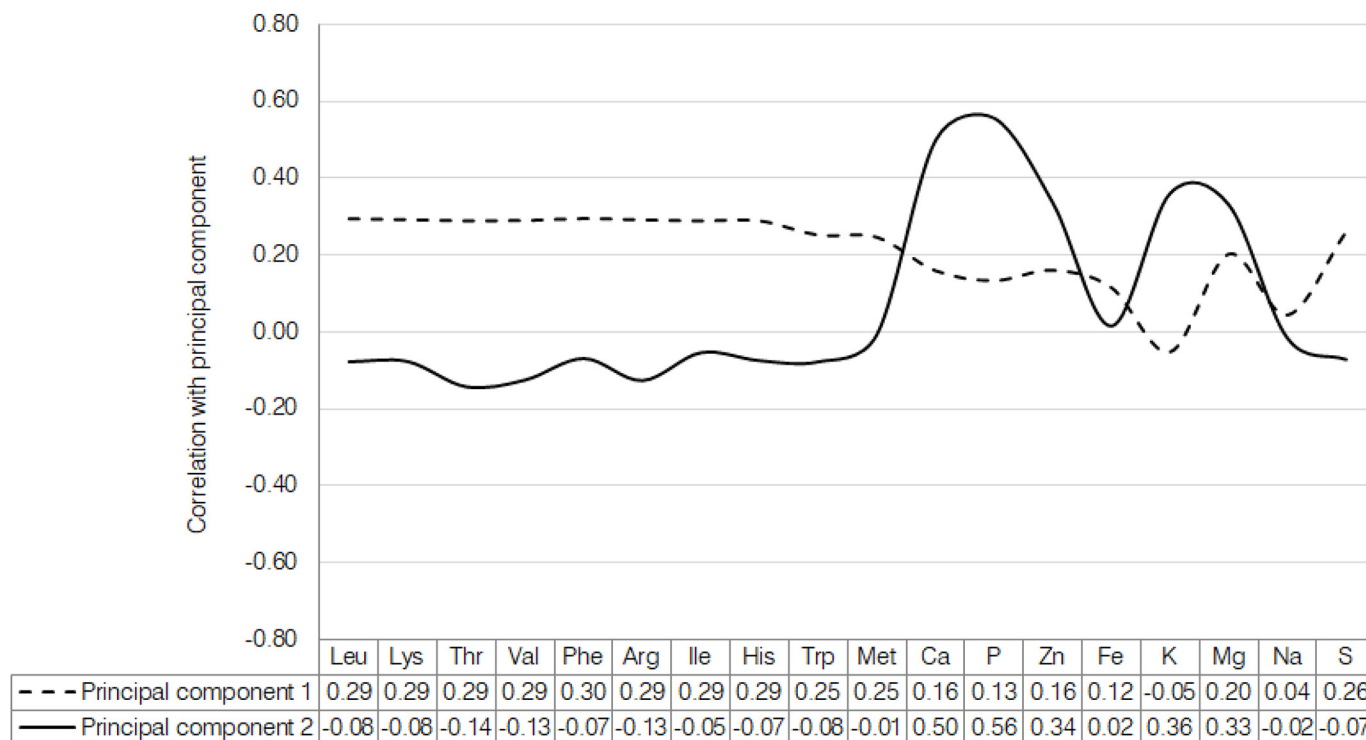


Figure 4. Correlations calculated between the predictors (x-axis) and the first 2 principal components.

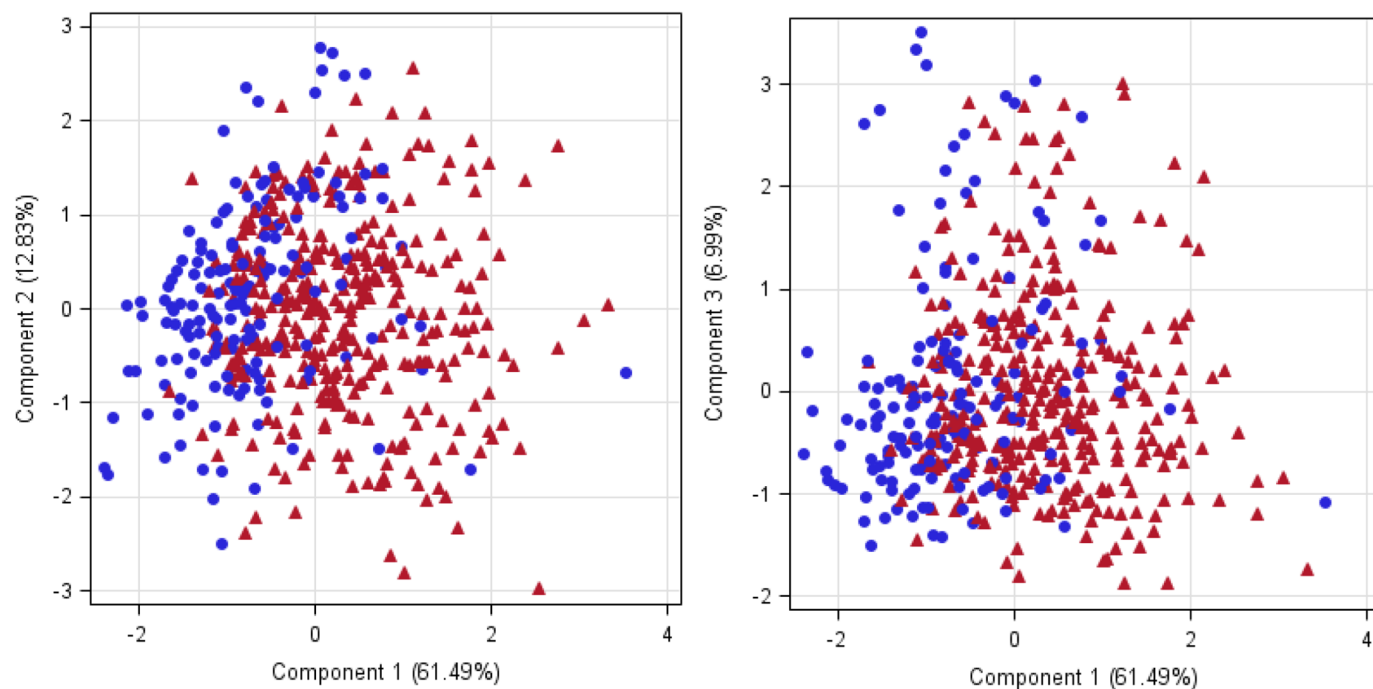


Figure 5. Graphical output of the scores of the principal components. Blue dots and red triangles represent low-quality ($\text{IgG} < 70 \text{ g/L}$; $n = 159$) and good-quality ($\text{IgG} \geq 70 \text{ g/L}$; $n = 349$) samples, respectively.

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The ability of a handheld near-infrared spectrometer to do a rapid quality assessment of bovine colostrum, including the immunoglobulin G concentration

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The ability of a handheld near-infrared spectrometer to do a rapid quality assessment of bovine colostrum, including the immunoglobulin G concentration

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ABSTRACT

Portable infrared-based instruments have made important contributions in different research fields. Within the dairy supply chain, for example, most of portable devices are based on near-infrared spectroscopy (NIRS) and are nowadays an important support for farmers and operators of the dairy sector, allowing fast and real-time decision-making, particularly for feed and milk quality evaluation and animal health and welfare monitoring. The affordability, portability, and ease of use of these instruments have been pivotal factors for their implementation on farm. In fact, pocket-sized devices enable nonexpert users to perform quick, low-cost, and nondestructive analysis on various matrixes without complex preparation. Because bovine colostrum (BC) quality is mostly given by the IgG level, evaluating the ability of portable NIRS tools to measure antibody concentration is advisable. In this study we used the wireless device SCiO manufactured by Consumer Physics Inc. (Tel Aviv, Israel) to collect BC spectra and then attempt to predict IgG concentration and gross and fine composition in individual samples collected immediately after calving (<6 h) in primiparous and pluriparous Holstein cows on 9 Italian farms. Chemometric analyses revealed that SCiO has promising predictive performance for colostral IgG concentration, total Ig concentration, fat, and AA. The coefficient of determination of cross-validation (R^2_{CV}) was in fact ≥ 0.75). Excellent accuracy was observed for dry matter, protein, and S prediction in cross-validation and good prediction ability in external validation ($R^2_{CV} \geq 0.93$; the coefficient of determination of external validation, R^2_v , was ≥ 0.82). Nonetheless, SCiO's ability to discriminate between good- and low-quality samples ($\text{IgG} \geq$ vs. < 50 g/L) was satisfactory. The affordable cost, the accurate predictions, and the user-friendly design, coupled with

the increased interest in BC within the dairy sector, may boost the collection of extensive BC data for management and genetic purposes in the near future.

Key words: colostrum, dairy cattle, portable instrument, spectroscopy, passive transfer of immunity

INTRODUCTION

In the dairy sector, infrared spectroscopy-based technologies are acknowledged to be moderately to highly accurate, have been widely accepted by stakeholders, and are implemented for various purposes. Inexpensive, sometimes portable, and easy-to-use infrared-based tools have boosted the improvement of knowledge and transition toward better management practices, including animal health and welfare monitoring. In the case of milk and dairy products, the spectral hotspots mostly belong to the near- (800–2,500 nm) and mid-infrared (2,500–25,000 nm) regions. If mid-infrared spectroscopy is used worldwide for the collection of milk composition traits to be used as phenotypes for milk payment and genetic evaluation, near-infrared spectroscopy (NIRS) is the most popular technology for rapid quality assessment of solid and semisolid material like feed and foodstuff (curd, cheese, yogurt, and so on). The repeatability, reproducibility, and overall accuracy of benchtop instruments is so good that NIRS has been recognized as a gold standard for gross composition by the International Organization for Standardization (ISO, 2020; ISO21543:2020) of milk and derived products for official use.

In recent years, portable NIRS instruments have been promoted under the umbrella of precision livestock farming, paving the way for rapid and customized decision-making on dairy farms (Evangelista et al., 2021; Pu et al., 2021). In fact, benchtop instruments, as well as the pocket-size devices, can ensure a rapid and nondestructive analysis of a variety of matrixes, which can be performed by nonexpert users after brief training. Furthermore, preparation of samples is minimal or even absent. In terms of accuracy, portable devices generally

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

perform worse than benchtop ones. This is the result of worse wavelength reproducibility, lower resolution, and reduced spectral range (Sun et al., 2020; Mishra et al., 2021), as well as the quality of pre-existing models already installed. Prediction accuracy of NIRS instruments is, in fact, related to the goodness of fit of the built-in equation(s) and representativeness of the set used for calibration, which have to be robust and developed in such a way as to avoid overfitting. The majority of microspectrometers, for example, SCiO (Consumer Physics Inc., Tel Aviv, Israel), NIRONE 2.2 (Spectral En109 gines, Finland), or F750 (Felix Instrument, Camas, WA), are designed to allow technicians to develop and install customized prediction models (Ryckewaert et al., 2022). Moreover, they are specifically designed to be suitable for operation by nonexpert users, such as farmers, through predeveloped software and applications that ensure ease of use (Beć et al., 2020). At the same time, NIRS predictions are directly affected by the precision of the reference method used (Sørensen, 2002; Blanco and Peguero, 2010) and by the intrinsic characteristics of the samples undergoing analysis. In fact, the most reliable outcomes are obtained under standard conditions, that is, in the presence of appropriate sample collection, manipulation, and scanning following the manufacturer's instructions.

Given its importance for neonatal calves and its recent industrial applications, bovine colostrum (BC) currently represents one of the most interesting semisolid matrixes to be analyzed with low-cost portable tools within the whole dairy supply chain, that is, not only at farm level (Costa et al., 2023). The BC composition is highly variable in the first hours after calving; in particular, this is the case for the narrow-sense quality of BC, which is given by the concentration of Ig, particularly of the isotype IgG. Although for different reasons, from both the producers' and farmers' standpoints, IgG is the most important criterion for evaluating BC quality. In fact, a high concentration of IgG in BC, coupled with good feeding and management practices, reduces the risk of passive transfer failure in neonatal calves. At the same time, commercial manufacturers of food or pharmaceutical items containing BC are willing to pay more if the concentration of antibodies in the supplied volume is good (Costa et al., 2023). Conventionally, veterinarians and farmers consider BC with IgG < 50 g/L to be of insufficient quality for feeding calves, especially at their first meal. This threshold has international validity (Godden et al., 2019). To optimize calves' survival in a precision feeding scenario, the possibility of measuring the IgG level of either pooled or individual BC with portable NIRS tools becomes interesting.

Therefore the aim of the present study was to evaluate the predictive ability of a wireless handheld NIRS

(SCiO), for evaluation of BC IgG concentration, and both gross and fine composition, through home-made equations. The possibility of recording sufficiently accurate IgG data in the field would, in fact, improve the knowledge about farm BC quality, creating the basis for the definition of strategic precision feeding in nonsuckling calves.

MATERIALS AND METHODS

Sampling

Ethical approval was not required for the present study per institutional guidelines and local legislation because only farmers interacted with the cows. The animals' owners involved in the study signed a written informed consent, joined the experiment on a voluntary basis, and were associates of the Veneto Region Breeders Association (ARAV, Vicenza, Italy). Individual samples from 709 purebred Holstein cows, 215 primiparous and 494 pluriparous, that calved between spring 2019 and spring 2020 were collected from 9 commercial farms located in Northeast Italy as described by Costa et al. (2022) and Goi et al. (2023a). Overall, the selected farms were characterized by an intensive farming system, freestall barns, TMR administration, twice-a-day milking, and no access to pasture. The experimental design covered the variability of calving season and parity order, which ranged from 1 to 8 and averaged 2.47 ± 1.42 lactations. In line with the experimental protocol, calves were separated from dams immediately after birth, suckling was absent, and only the first colostrum (120 mL) was collected from each cow between 0 and 6 h after calving. The tubes used were sterile, made of polypropylene, and free of preservative (SMIPA srl, Vicenza, Italy). Once filled, the tubes were stored at -20°C (Costa et al., 2021a). Vaccination before calving against rotavirus, coronavirus, and *E. coli* was not performed in the donor cows to avoid presence of hyperimmune colostrum (Dunn et al., 2017; Costa et al., 2023).

Reference Analyses

The procedure carried out to perform the reference analyses is the same as described by Goi et al. (2023a,b) following the workflow illustrated in Figure 1. Briefly, protein was measured by using the Kjeldahl method 991.20 (AOAC International, 2000), fat content was determined according to Verbands Deutscher Landwirtschaftlicher Untersuchungs und Forschungsanstalten (VDLUFA) VI C15.2.1 method (VDLUFA, 2013), and lactose was quantified through HPLC (Aminex HPX 87H column, 300 mm $\times$ 7.8 mm; Bio-Rad), with lactose concentration determined using ChromNAV ver. 2.0 (Jasco).

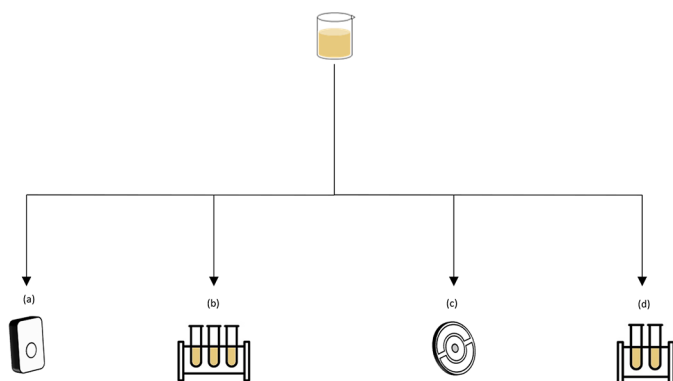


Figure 1. Workflow adopted according to the destination of the colostrum samples: (a) spectra collection through the SCiO spectrometer, (b) gross composition analyses with gold standards, (c) determination of Ig isotype concentrations via radial-immunodiffusion assay, and (d) quantification of mineral and AA content.

The concentration of Ig (isotypes G, A, and M) was assessed using the bovine-specific radial-immunodiffusion (RID) assay purchased from Triple J Farms (Bellingham, WA), whose repeatability has been preliminarily tested by intra- and interassay CV, as described in detail by Costa et al. (2021b) and Goi et al. (2023a). The total Ig concentration (**IgTot**) was calculated as the sum of all isotypes exclusively for the samples with the 3 concentrations known.

Following the procedure already described by Goi et al. (2023b), for mineral quantification, a 2-mL aliquot was mineralized and then analyzed using inductively coupled plasma-optical emission spectrometry (Spectro Arcos, Spectro Analytical Instruments GmbH, Kleve, Germany), whereas for AA quantification, a 15-mL aliquot of each sample was lyophilized, underwent acid hydrolysis and precolumn derivatization, and was separated by a reversed-phase HPLC and analyzed by UV detection.

Development of NIRS Models

Spectra of BC were recorded through SCiO, which works in reflectance mode in the wavelength region between 740 and 1,070 nm, at intervals of 1 nm. For this purpose, for each sample 50 mL of thawed BC was put in a sterile tube and left at room temperature for 1 h to standardize scanning conditions. Subsequently, before the scan, the tube was inverted to homogenize the sample. The SCiO was equipped with a manufacturer-provided adapter featuring a ceramic bottom to scatter back the light emitted from the source toward the detector while passing through the matrix. This was inserted directly into the test tube to record the spectra in transreflectance (**Transfl**) mode. The instrument was also cleaned with

sterile water and dried with a disposable towel between each sample.

For each sample, 3 spectra were stored and then the average was calculated. Spectral data points were converted from **Transfl** to absorbance (**Abs**) using the formula: $Abs = \log_{10}(1/Transfl)$ and then used for the calibration once paired with the reference data. Reference outliers (values exceeding ± 3 SD from the mean) and spectral outliers (Mahalanobis distance > 3) were discarded before developing calibration models. Data points of all the traits investigated were normally distributed. The only exception was Fe, which was thereby logarithmically transformed before the statistical analysis.

For all the traits, chemometric analyses were performed using WinISI 4.10 software (Infrasoft International, Port Matilda, PA) through a modified partial least squares regression algorithm. Preliminarily, different scatter corrections were tested on spectral data: none, detrend, standard normal variate, standard normal variate and detrend, and multiplicative scatter correction. Each of the mentioned pretreatments was tested in combination with mathematical treatments: 0,0,1,1; 1,4,4,1; 1,5,5,1; 1,8,8,1; 2,5,5,1, where the first digit is the derivative, the second digit is the gap over which the derivative was calculated, the third digit is the gap for the first smoothing, and the fourth digit is the gap for the second smoothing. Three steps of outliers elimination were performed after each modified partial least squares round ($n = 3$), at the end of which the samples whose predicted value differed by more than $\pm 3 SE_{CV}$ ($T\text{-statistic} > 3$) from the reference value were excluded.

The entire dataset was first divided for each trait to create the training set, selecting randomly 75% of data (= of cows), with the remaining 25% representing the external validation, or testing, set. The means and SD of the 2 sets were confirmed to be similar. The prediction equations were developed on the training set and internally tested by performing a 5-fold cross-validation. To avoid overfitting, the number of latent variables (LV), which minimized the root-mean-square error of cross-validation, were included in the model. In terms of predictive performance, the model with the lower standard error of cross-validation and the greater coefficient of determination of cross-validation (R^2_{CV}) was elected as the best and underwent external validation. After that, for each trait the final model was applied to the external samples (testing set) where the reference values were masked. Performance in external validation was evaluated based on the greater coefficient of determination of external validation (R^2_V) and the greater residual predictive deviation (**RPD**). Residuals normality was inspected and the TTEST procedure of SAS v. 9.4 (SAS Institute Inc., Cary, NC) was adopted to test that the bias did not statistically differ from zero.

In addition to regression, a discriminant analysis was used on the entire set net of outliers to evaluate the classification ability of SCiO based on spectral wavelengths of BC. For this purpose, the STEPDISC procedure of in SAS v. 9.4 (SAS Institute, Cary, NC) was used for a preliminary backward elimination of spectral regions. This step was followed by the DISCRIM procedure where “low-quality” and “good-quality” samples were identified using 3 different IgG thresholds: 50, 60, and 70 g/L. The model performance included the specificity, the sensitivity, the overall percentage of samples correctly classified, and the Matthews correlation coefficient (MCC), which was calculated based on the number of true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN) as follows:

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}}.$$

Diagnostic Accuracy

The receiver operating characteristic curve was used to evaluate the diagnostic accuracy of the predicted IgG for the external validation set by using LOGISTIC procedure of SAS v. 9.4 (SAS Institute, Cary, NC). The same 3 thresholds used for the discriminant analysis were adopted and the area under the curve (AUC; Šimundić, 2009) was used to evaluate the classification ability of the infrared-predicted IgG.

RESULTS AND DISCUSSION

In the present study, the predictive ability of SCiO for IgG and BC composition traits was tested using samples ($n = 709$) collected from 9 farms. The BC collection was done by the farm staff, preliminarily trained, using the same protocol and within 6 h from calving.

Because the reference data was derived from various analyses, that is, different kits, devices, or procedures, the final number of records available differed slightly across traits. Table 1 shows the number of samples with the matching reference value, spectrum, and net of outliers. In the case of RID 3 kits were available, one for each isotype. The final number of records for IgG, IgA, IgM, and IgTot (Table 1) differed because samples may have been excluded due to nonreadable and noncircular RID rings, final calculated concentrations out of the kit detection range, and missing or lost aliquots (Costa et al., 2021b).

Descriptive statistics confirm that IgG was the predominant fraction and was highly variable, ranging between 0.68 and 198.90 g/L. Nevertheless, this fraction was less

variable compared with the other 2 isotypes. Overall, 75 samples were considered to be of insufficient quality, having IgG < 50 g/L, thus the vast majority presented a concentration above the conventional cut-off (Godden et al., 2019). Regarding gross composition, descriptive statistics are in line with the findings of Gopal and Gill (2000) and Godden (2008). The highest CV was that of fat (67%) and the lowest (<19%) was that of ash and DM. The average fat content (4.64%; Table 1) was in agreement with Elfstrand et al. (2002; pooled samples from Swedish Friesian cows) but slightly lower than the average content of 6.70% reported by Kehoe et al. (2007) on individual BC samples collected within 4 h from calving in 55 cows. As reviewed by Costa et al. (2023), when comparing BC of different studies, it is important to point out that dissimilarities may be due to the collection time interval after birth and the gold standard used for the Ig analysis.

Distribution of AA and mineral values for each parameter was normal except for Fe (Table 1), which was logarithmically transformed before performing the chemometric analysis. The most and least abundant AA were Leu (294.95 ± 121.51 mg/100 g as is) and Met (48.23 ± 20.91 mg/100 g as is), respectively. The ranking of AA in terms of concentration was the same as the one reported by Puppel et al. (2019) for BC, except for Arg, whose concentration in the cited study was greater than Met. Small differences in terms of variability were noticed among the AA analyzed; their CV ranged from 39.89% (Ile) to 45.44% (Thr). For the minerals, Ca and P were the most abundant with concentrations of approximately 2 g/kg (as is) of BC, followed by S and Zn whose concentrations were approximately 1.4 g/kg as is (Table 1). Minerals present in low quantity were Na and Mg, followed by the 2 minor minerals Zn and Fe. In general, the mineral content of BC in this study resembled the values reported by Playford and Weiser (2021) for Ca, P, K, Na, Mg, and Zn. In contrast, Kehoe et al. (2007), using BC from 55 cows, found greater average mineral concentrations compared with this study, that is, the averages of Ca, P, S, K, Na, Mg, Zn, and Fe were 4,716.10; 4,452.10; 2,595.67; 2,845.89; 1,058.93; 733.24; 38.10; and 5.33 mg/kg, respectively, as is.

In the present study, the CV of major minerals was generally lower than that of AA, ranging from 14.87% (K) to 27.42% (Mg). In contrast, minor minerals like Zn and Fe varied widely (CV of 36.51% and 46.45%, respectively). As indicated for gross composition, it is often difficult to fairly compare BC components of different studies. The concentration of fine composition traits like minerals and AA is known to be strongly affected by the sampling protocol used, feeding and husbandry system, and parity and breed of the donor cow (Costa et al., 2021b).

Table 1. Descriptive statistics of bovine colostrum Ig¹ fractions, composition traits, AA, and mineral concentrations measured with the reference method after outlier elimination

Trait	n	Mean	SD	Minimum	Maximum	CV, %
Major compound						
IgG, g/L	647	91.97	35.37	0.68	198.90	38.45
IgA, g/L	555	4.65	2.73	0.13	13.95	58.65
IgM, g/L	632	5.08	2.42	0.18	11.89	47.65
IgTot, g/L	554	102.88	36.00	17.96	196.86	34.99
DM, %	685	24.04	4.52	10.30	36.61	18.82
Protein, %	688	14.49	3.73	4.26	25.22	25.72
Fat, %	679	4.64	3.10	0.12	14.67	66.79
Ash, %	683	1.14	0.18	0.63	1.68	15.58
Lactose, mg/100 mg	701	2.37	0.53	0.74	4.06	22.34
AA, mg/100 g as is						
Leu	684	294.95	121.51	20.38	670.74	41.20
Lys	681	281.45	116.26	20.42	646.55	41.31
Thr	683	225.94	102.66	12.26	546.76	45.44
Val	682	189.07	82.32	12.63	429.99	43.54
Phe	682	139.32	55.83	10.04	312.12	40.07
Arg	681	129.14	56.87	6.71	292.56	44.04
Ile	681	104.34	41.62	9.14	232.46	39.89
His	684	96.88	40.23	4.45	223.24	41.53
Trp	681	69.00	31.08	4.14	165.40	45.04
Met	682	48.23	20.91	5.10	111.43	43.35
Mineral, mg/kg as is						
Ca	683	2,188.94	520.64	631.48	3,694.25	23.79
P	684	1,957.27	398.75	835.12	3,113.15	20.37
S	686	1,445.68	380.05	403.91	2,533.64	26.29
K	684	1,420.99	211.32	729.96	2,068.66	14.87
Na	678	557.51	144.29	250.39	1,082.69	25.88
Mg	681	339.08	92.96	107.13	637.18	27.42
Zn	681	20.28	7.41	2.86	42.23	36.51
Fe	672	1.37	0.64	0.32	5.09	46.45
Fe ²	672	0.10	0.17	-0.50	0.71	172.17

¹IgG, IgA, IgM, and their sum (IgTot).²Log transformed.

SCiO for Ig and Gross Composition

The advantages and applications of a rapid and inexpensive assessment of BC composition through infrared spectroscopy using benchtop instruments have been already discussed in literature (Navrátilová et al., 2006; Rivero et al., 2012; Franzoi et al., 2022; Goi et al., 2023a; Goi et al. 2023b). However, only few studies have investigated the application of portable devices for analysis of mammary gland secretions, either BC or milk (Guerra et al., 2024). Although portable devices have been used to detect adulterants in milk and to assess milk fat and fatty acids content (Santos et al., 2013; Amr et al., 2018; Llano Suárez et al., 2018), none has demonstrated doable and useful applications on BC so far.

Portable spectrometers can be used online and save time when it comes to visualizing and interpreting outputs of the analysis. Obtaining real-time information on mammary gland secretions composition allows the farmer to evaluate the quality of the freshly collected BC or milk and immediately determine the most appropriate destination. In the case of BC, for example, banking is a popular and highly recommended management strategy,

especially in well managed herds. Having the ability to determine the narrow-sense quality of BC to be stored is pivotal for calves' health and precision feeding. Potentially, infrared analysis can be performed quickly on the BC produced at first and subsequent milkings (e.g., transition milk).

The average raw absorbance spectra (740–1,070 nm, along with the SD) of BC is depicted in Figure 2. The pattern presents a weak peak around 840 nm and a broad peak around 980 nm. The former is due to a combination of C–H vibrations and can be ascribed to any of the main milk components, whereas the latter arises from the symmetric and asymmetric vibration of water (Šašić and Ozaki, 2001). As indicated by the dashed lines, a slightly wider variation was observed for the water absorption band, likely due to the difference in solids content, especially lipids. As explained above, the BC fat content was much more variable than other constituents and can cause a shift in the spectrum (Rivero et al., 2012).

Tables 2 and 3 report the predictive performances of the developed equations in cross and external validation, respectively, for the Ig fractions, gross composition, AA, and minerals, along with the number of samples used in

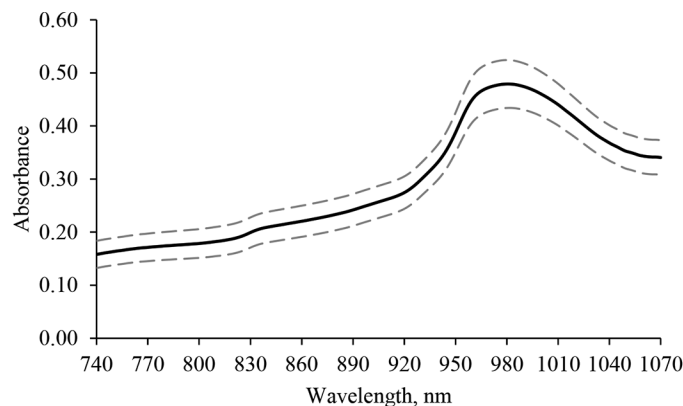


Figure 2. Average raw spectrum ($n = 709$, solid line) obtained using the SCiO spectrometer. Dashed lines indicate the phenotypic variability observed (± 1 SD from the mean).

the final models. Overall, considering the Ig isotypes and the gross composition, the outliers eliminated were $\leq 12.1\%$, with the exception of fat, which suffered a slightly higher elimination of samples (14.7%). Outliers can be either in spectral data, chemical (reference) values, or both (Wang et al., 2018). In the case of BC, the number of outliers detected could be attributed to the viscosity of the matrix, which likely influenced the reflection of light on the ceramic surface of the adapter used in scanning the samples with the instrument (Williams, 2007). In the case of cross-validation of IgG (Table 2), for example, 34 of the 38 discarded samples had an outlier spectrum. Performing cross-validation on the entire data set, the number of LV oscillated between 5 and 10 and the most-used scatter correction was detrend along with first derivative. In contrast, the range of LV used for the external validation was almost the same as in the cross-validation, being between 4 and 10, but the accuracy is slightly changed as expected (Table 3). In this case the standard normal variate alone or with detrend was the most frequently used correction, with first or second derivative. In this study, SCiO demonstrated its ability to predict BC composition. In particular, according to the classification of R^2 and RPD made by Williams (2003, 2014), an approximate and sufficient ability to quantify IgG ($R^2_{CV} = 0.78$; RPD = 2.1) was observed in cross-validation (Table 2). The same can be said for IgTot (Table 2). More precisely, the formal interpretation of the RPD suggests that the models have the potential for qualitative evaluation or rough screening solely (Williams, 2003, 2014), therefore limiting the potential of SCiO for the IgG concentration assessment of such an important mammary gland secretion. Thus, considering the matrix's relevance on the farm for good calf management and having in mind its complexity (e.g., viscos-

ity) per se, achieving that level of accuracy in practice is deemed sufficient also for compounds quantification (Williams, 2003, 2014). Moreover, attention should be given to the fact that pocket-sized instruments are designed to accept a relatively limited overall performance compared with benchtop ones, but with a benefit in terms of cost-effectiveness. Therefore, the accuracy of models obtained with spectral data from portable instruments may often not coincide and be inferior to that of laboratory instruments, but this is a necessary compromise to tailor the instruments to their specific field of application (Beć et al., 2022), in this particular case for use by private practitioner veterinarians or farmers.

Likely due to their low concentrations compared with IgG and IgTot, both IgA and IgM did not achieve a satisfactory prediction accuracy ($R^2_{CV} < 0.66$). The result for IgG is almost the same as that obtained by Elsohaby et al. (2018) with laboratory mid-infrared spectroscopy analysis on 60 cows within 1 h of calving, but lower than the accuracy reported by Rivero et al. (2012) scanning colostrum samples with the NIR Systems 6500 monochromator, a benchtop instrument. The performance related to the prediction of Ig remained unchanged when validating the models on the validation subset, with sufficient accuracy for an approximate quantification of the IgG ($R^2_V = 0.80$) and IgTot content ($R^2_V = 0.70$), but was not satisfactory for the other 2 fractions (Table 3). This indicates that the developed models were able to be applied to samples with unknown values without noticing a change in precision. Also Franzoi et al. (2022), predicting IgG content using the DS2500 near-infrared benchtop spectrometer (400–2500 nm; FOSS, Hillerød, Denmark) showed no significant changes in prediction accuracy between cross and external validation. Dry matter and protein content achieved excellent prediction accuracy in cross-validation, with $R^2_{CV} \geq 0.95$ and RPD ≥ 4.4 , and this promising performance was in agreement with the correlation coefficients reported by Navrátilová et al. (2006) for BC (Table 2). In that study, a benchtop NIRS instrument was used to evaluate its predictive ability scanning 90 colostrum samples collected from 18 cows over a wide time interval. A slight decrease in the accuracy was detected when externally validating the performances of the models ($R^2_V \geq 0.89$ and RPD ≥ 3.1), nevertheless indicating the adequacy of the predictive model to perform a quality control with a punctual determination of the traits (Table 3). Unfortunately, Navrátilová et al. (2006) did not perform external validation to compare the data. Although the model developed for fat presented a lower accuracy compared with the other traits and compared with Navrátilová et al. (2006), still the R^2_{CV} (0.86) and RPD (2.7) guarantee a satisfactory prediction. When applying an external validation, fat experienced a signifi-

Table 2. Fitting statistics¹ of modified partial least square regression analysis in 5-fold cross-validation for bovine colostrum Ig, ² gross composition, AA, and minerals (% of kg as is) on the entire set

Trait	Sc-Mt ³	Out	LV	SE _C	R ² _C	SE _{CV}	R ² _{CV}	RPD
Major compound								
IgG, g/L	D-2,5,5,1	5.9	6	15.74	0.80	16.50	0.78	2.12
IgA, g/L	D-1,4,4,1	12.1	6	1.77	0.48	1.84	0.44	1.33
IgM, g/L	D-1,8,8,1	7.6	5	1.80	0.43	1.82	0.41	1.30
IgTot, g/L	None-1,5,5,1	9.9	7	17.30	0.76	17.64	0.75	2.02
DM, %	D-2,5,5,1	9.8	7	0.92	0.95	0.97	0.95	4.41
Protein, %	None-2,5,5,1	7.3	10	0.72	0.96	0.78	0.96	4.72
Fat, %	SNV-1,4,4,1	14.7	5	1.06	0.87	1.08	0.86	2.68
Ash, %	SNVD-0,0,1,1	10.7	7	0.14	0.30	0.15	0.27	1.17
Lactose, mg/100 mg	D-1,8,8,1	11.4	9	0.27	0.68	0.29	0.65	1.69
AA, mg/100 g as is								
Leu	SNVD-1,5,5,1	11.0	7	44.09	0.85	45.63	0.84	2.59
Lys	D-2,5,5,1	7.2	8	45.51	0.83	47.35	0.82	2.43
Thr	SNV-1,4,4,1	11.0	7	38.77	0.84	39.71	0.83	2.49
Val	MSC-1,4,4,1	12.5	7	31.09	0.84	31.69	0.83	2.46
Phe	SNV-1,5,5,1	11.6	8	19.63	0.86	20.07	0.86	2.69
Arg	MSC-1,4,4,1	11.6	8	20.95	0.85	21.78	0.84	2.57
Ile	MSC-1,5,5,1	11.2	8	17.05	0.81	17.55	0.80	2.32
His	MSC-1,4,4,1	11.8	7	15.82	0.82	16.13	0.82	2.39
Trp	D-0,0,1,1	10.1	11	12.50	0.82	12.98	0.81	2.38
Met	MSC-1,5,5,1	10.6	9	9.42	0.77	9.81	0.75	2.10
Mineral, mg/kg as is								
Ca	MSC-1,4,4,1	9.22	6	403.28	0.37	412.68	0.34	1.24
P	SNV-1,5,5,1	9.94	5	316.79	0.34	320.57	0.32	1.21
S	D-2,5,5,1	8.75	7	92.80	0.94	97.41	0.93	3.80
K	MSC-0,0,1,1	20.03	6	189.58	0.10	192.23	0.07	1.04
Na	SNV-1,8,8,1	17.11	6	118.98	0.12	121.11	0.09	1.05
Mg	MSC-1,4,4,1	10.28	6	65.02	0.46	66.11	0.44	1.34
Zn	SNV-1,5,5,1	9.84	6	5.49	0.41	5.61	0.38	1.27
Fe ⁴	None-2,5,5,1	10.57	9	0.10	0.50	0.11	0.43	1.33

¹Sc-Mt = scatter correction – mathematical treatment; Out = percentage of outliers; LV = latent variable; SE_C = standard error of calibration; R²_C = coefficient of determination of calibration; SE_{CV} = standard error of cross-validation; R²_{CV} = coefficient of determination of cross-validation; RPD = residual predictive deviation.

²IgG, IgA, IgM, and their sum (IgTot).

³D = detrend; MSC = multiplicative scatter correction; None = no scatter correction; SNV = standard normal variate; SNVD = SNV + detrend.

⁴Log-transformed.

cant reduction in the R²_V and RPD values (0.54 and 1.3, respectively), indicating that the model cannot provide a robust prediction (Williams, 2003, 2014). The scarce prediction performance can be attributed to the high variability of the reference data; the high content of solids in BC and the dilution may have affected the spectra. The rather low accuracy of lactose prediction indicates that only a discrimination between low and high concentration could be made (R²_{CV} = 0.65; R²_V = 0.61), whereas ash content did not achieve sufficient accuracy in either cross (R²_{CV} = 0.27) and external validation (R²_V = 0.19). Despite this, implementation of SCiO in the field is not or only marginally affected. In fact, still the most important parameter for the narrow-sense BC quality remains the IgG. This is true for both farmers and manufacturers interested in using BC as ingredient for industrial purposes (Costa et al., 2023). However, apart from the IgG, assessment of the gross composition would allow the real-time adjustment of the BC administered to calves, especially under a precision feeding scenario.

Prediction of Fine Composition Traits

To the authors' knowledge no studies have attempted to predict essential AA and minerals content of BC using portable NIRS devices (Costa et al., 2023). However, with fine composition we refer to traits important for the broad-sense quality of BC, including substances fundamental for the neonate growth and health. According to the results, it is possible to predict AA and minerals content; performance obtained in cross-validation on the total data set and in external validation on the data set divided into training and testing sets are summarized in Tables 2 and 3, respectively. The outliers detected during the calibration process were below 13% for all the traits, except for K and Na. The number of LV of the best prediction models ranged between 5 and 11 in cross-validation and between 2 and 10 in external validation. The most applied pretreatment was multiplicative scatter correction for AA and standard normal variate for minerals, both preferring first rather than second order of derivatization. Most of

Table 3. Fitting statistics¹ of modified partial least square regression analysis in external validation for bovine colostrum Ig,² gross composition, AA, and minerals

Trait	Sc-Mt ³	n _{ext}	LV	SE _v	R ² _v	RPD
Major compound						
IgG, g/L	D-1,4,4,1	150	10	16.05	0.80	2.22
IgA, g/L	SNV-1,5,5,1	134	7	1.94	0.46	1.41
IgM, g/L	SNV-1,8,8,1	149	5	1.94	0.37	1.25
IgTot, g/L	None-2,5,5,1	137	4	19.98	0.70	1.82
DM, %	D-2,5,5,1	169	6	1.51	0.89	3.07
Protein, %	None-1,5,5,1	163	10	1.10	0.92	3.39
Fat, %	SNVD-2,5,5,1	170	4	2.43	0.54	1.28
Ash, %	MSC-2,5,5,1	171	5	0.16	0.19	1.10
Lactose, mg/100 mg	D-0,0,1,1	167	10	0.33	0.61	1.65
AA, mg/100 g as is						
Leu	SNV-1,4,4,1	168	7	58.82	0.77	2.06
Lys	SNVD-0,0,1,1	162	10	62.15	0.72	1.90
Thr	SNV-1,4,4,1	164	7	43.94	0.82	2.34
Val	SNVD-1,8,8,1	171	7	47.15	0.69	1.77
Phe	SNV-1,4,4,1	165	7	24.05	0.82	2.34
Arg	SNV-2,5,5,1	163	5	27.51	0.76	2.10
Ile	MSC-1,8,8,1	161	9	21.49	0.73	1.97
His	D-2,5,5,1	165	5	20.16	0.73	1.99
Trp	D-2,5,5,1	168	4	15.61	0.75	2.03
Met	SNV-1,4,4,1	164	8	11.01	0.72	1.92
Mineral, mg/kg as is						
Ca	None-1,5,5,1	162	6	444.37	0.26	1.18
P	SNVD-0,0,1,1	162	7	354.18	0.25	1.13
S	D-1,4,4,1	163	7	166.10	0.82	2.31
K	MSC-2,5,5,1	167	2	214.68	0.03	0.99
Na	MSC-0,0,1,1	170	7	145.92	0.04	1.00
Mg	MSC-2,5,5,1	171	4	71.02	0.44	1.33
Zn	SNVD-1,4,4,1	171	6	6.23	0.34	1.21
Fe ⁴	SNV-2,5,5,1	163	6	0.14	0.36	1.25

¹Sc-Mt = scatter correction – mathematical treatment; n_{ext} = number of samples used to perform the external validation net of spectral outliers; SE_v = standard error of external validation; R²_v = coefficient of determination of external validation; RPD = residual predictive deviation.

²IgG, IgA, IgM, and their sum (IgTot).

³D = detrend; MSC = multiplicative scatter correction; None = no scatter correction; SNV = standard normal variate; SNVD = SNV + detrend.

⁴Log-transformed.

the AA obtained good prediction accuracy when cross-validation was performed ($R^2_{CV} \geq 0.82$; Williams, 2003); in particular, Phe, Leu, Arg, and Thr reached a $RPD \geq 2.5$, which represents the cut-off to consider a calibration model suitable for application in the field as a screening method (Williams, 2014). At the same time, it is worth considering that the conventional RPD classification is not adapted to the dairy sector. In fact, as reported by Grelet et al. (2021), even models showing RPD below 2.5 can have certain potential. In dairy, the high cost of reference analyses and the difficulty of obtaining a large amount of robust phenotypes should be considered when evaluating infrared prediction models.

Using milk, McDermott et al. (2016) predicted the Lys, Val, and Arg content via mid-infrared spectroscopy, which resulted in low correlations in cross-validation (0.69, 0.69, and 0.66, respectively). As expected, all AA suffered a slight reduction in accuracy when the models were applied to samples whose composition was masked. Threonine, Phe, Arg, Leu, Trp, and His maintained a level

of accuracy sufficient to be used in a sample screening perspective ($RPD \geq 2$), but the application of the equations for other AA is not recommended for application on external data sets.

Among minerals, only the equation for S achieved excellent precision in predicting the reference value, which allowed us to apply the model in quality control ($R^2_{CV} = 0.93$; $RPD = 3.8$). This is likely due to the presence of S in the structure of AA, making easy its identification with NIRS. In fact, only substances linked to organic molecules or associated with hydrated inorganic substances can be identified and predicted with infrared technologies (Clark et al., 1987). Therefore, minerals can be detected when they are found in organic complexes or chelates, and this is the case of the sulfhydryl groups found in proteins (Büning-Pfaue, 2003). All the other minerals considered in the study obtained an insufficient prediction ($R^2_{CV} < 0.45$), which could again be related to the inorganic form or the low concentration in the samples. Soyeyrt et al. (2009) obtained more accurate prediction

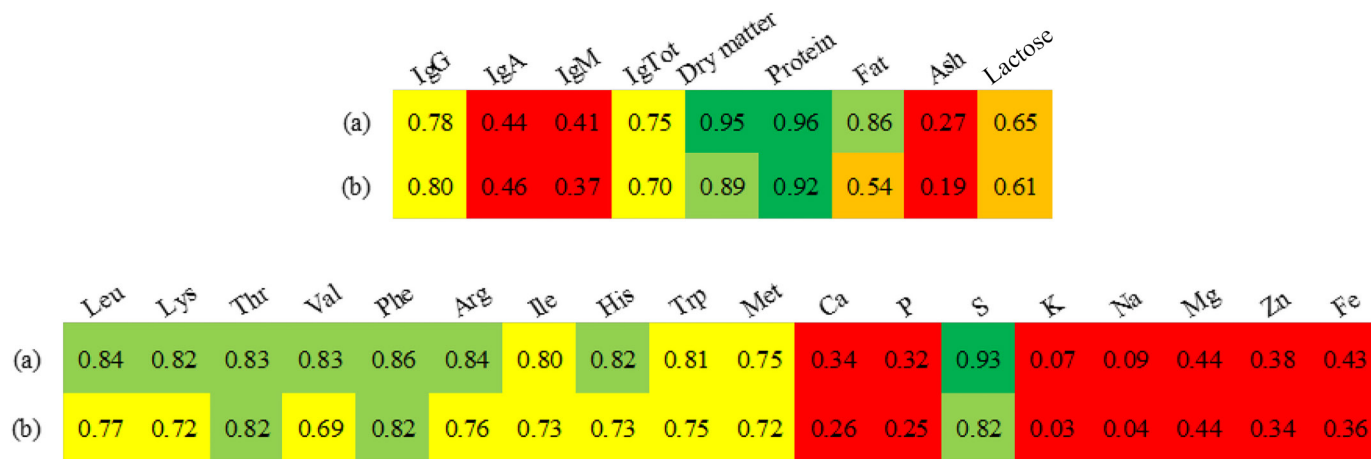


Figure 3. Traffic light classification of predictive performance in (a) cross-validation and (b) external validation for the colostrum traits analyzed. The classification is based on ranges given by Williams (2003): coefficient of determination <0.50 (red; not recommended), 0.50–0.65 (orange; exclusively for detection of extreme values or comparison of groups), 0.66–0.81 (yellow; approximate screening), 0.82–0.90 (light green; good quantitative screening), or >0.91 (dark green; excellent).

models for Ca, P, K, Mg, and Na, being satisfactory for Ca and P ($R^2_{CV} > 0.82$); however, they used milk spectra collected in the mid-infrared regions. Also, Visentin et al. (2016) predicted milk minerals content using mid-infrared spectroscopy without reaching successful accuracy ($R^2_{CV} \leq 0.62$). External validation for minerals showed an unsatisfactory precision ($R^2_V < 0.45$), except for S, which despite having undergone a reduction in accuracy, remains the only element that can still be estimated with the portable spectrometer for screening ($R^2_V = 0.82$; RPD = 2.3). Figure 3 provides a visual representation of the accuracy changes for all the predicted traits between cross-validation (R^2_{CV}) and external validation (R^2_V) using a traffic light classification system. Residuals of all prediction equations were normally distributed, and bias did not differ statistically from zero.

Although applications of portable NIRS instruments for the analysis of milk have been described in the literature (de la Roza-Delgado et al., 2017; Modroño et al., 2017; Guerra et al., 2024), a direct comparison is not recommended because the spectral ranges on which the samples were scanned do not coincide due to the large number of tools present in the field or tested under experimental conditions. For instance, de la Roza-Delgado et al. (2017) collected spectra of raw milk from reflectance spectra between 1,600 and 2,400 nm, whereas Modroño et al. (2017) used a miniaturized spectrometer working in the range of 910 to 1,676 nm. In any case, the suitability of portable devices for BC quality discrimination and prediction of AA and minerals was investigated for the first time in the present study.

The procedure to evaluate the spectral ability to discriminate good- and low-quality samples was carried out on the entire set net of outliers, comprising 3 different

IgG concentrations, including 50 g/L, which is the concentration usually considered worldwide as the minimum for an adequate passive transfer of immunity in neonatal calves (Godden et al., 2019). The MCC was 0.25, 0.27, and 0.32 for IgG at 50, 60, and 70 g/L, respectively. In biochemistry, similar MCC values suggest that models are far from being considered as usable for punctual IgG prediction; however, MCC ranges from –1 to 1, and values >0 are indicative of a prediction in the correct direction (Chicco and Jurman, 2023). The confusion matrix, which describes the classification performance of SCiO for the total number of samples is shown in Table 4, along with specificity and sensitivity, which were equal to 61.33% and 74.48%, respectively, for 50 g/L, 62.16% and 71.83% for 60 g/L, and 67.05% and 68.37 for 70 g/L. In particular, when setting the cut-off at 50 g/L the “low-quality” samples accounted for 12% of all samples, and 4 wavelengths were retained, namely 740, 747, 939, and 1,010 nm. In other words, 46 “low-quality” samples and 426 “good-quality” samples were correctly classified. This threshold, however, was quite strict for this study due to the low amount of “low-quality” samples, those with IgG below 50 g/L. Therefore, higher cut-offs were tested, that is, at 60 and 70 g/L, for which the “low-quality” samples were 17% and 27%, respectively. When the threshold was set at 60 g/L, the wavelengths used were 740, 741, 939, and 1,010 nm and the model correctly discriminated 62.2% of “low-quality” samples and 71.8% of the “good-quality” ones. The discriminant model developed using the third cut-off (70 g/L) retained an additional wavelength (742 nm) compared with the previous cut-off and correctly identified 67.1% and 68.4% of the samples belonging to the “low-quality” and “good-quality” classes, respectively. The spectral

Table 4. Confusion matrix¹ for quality level (low vs. high) defined using different cut-offs of IgG (n = 647)

Predicted quality	Quality cut-off					
	50 g/L (actual)		60 g/L (actual)		70 g/L (actual)	
	Low	High	Low	High	Low	High
Low	<i>61.33%</i> n = 46	<i>38.67%</i> n = 29	<i>62.16%</i> n = 69	<i>37.84%</i> n = 42	<i>67.05%</i> n = 118	<i>32.95%</i> n = 58
High	<i>25.52%</i> n = 146	<i>74.48%</i> n = 426	<i>28.17%</i> n = 151	<i>71.83%</i> n = 385	<i>31.63%</i> n = 149	<i>68.37%</i> n = 322

¹Percentage of samples correctly classified are in italics.

regions with important signals for IgG concentration were located in regions known for the presence of water, protein, and fat (Šašić and Ozaki, 2001). Because absorptions of these components overlap (Aernouts et al., 2015), achieving a precise correlation between the peak at a specific wavelength and the concentration of a BC component is not feasible.

The accuracy, which is the overall proportion of samples correctly classified, was 68%, 67%, and 68% from the lowest to the highest threshold.

On-Farm Use

The receiver operating characteristic curves (Figure 4) demonstrate that SCiO predictions of IgG are very good for the classification of the quality of colostrum samples. The AUC values obtained using the external validation set are close to unity and indicate an excellent discriminant ability of the infrared-predicted IgG (Figure 4; Šimundić, 2009). For the less restrictive threshold, 70 g/L, the AUC was the lowest but still outperforming (0.911). As

demonstrated by Chicco and Jurman (2023), even if both AUC and MCC are informative in classification issues, they provide different information. In this study, in fact, AUC looked to be almost optimal (Figure 4), but the MCC calculated for the 3 IgG thresholds were rather far from 1 (perfect prediction), ranging from 0.25 to 0.37. Such a situation happens particularly when there is an imbalance between the 2 classes, that is, when a certain percentage of samples of the less represented class are misclassified. Although the effect of imbalanced classes is basically negligible for the AUC—which can still be very high and promising—it penalizes the MCC (Chicco and Jurman, 2023).

When used in the field, SCiO's performance can be affected by factors not related to the goodness of fit of the models installed. For the analysis of BC, in fact, a brief protocol should be designed to ensure the quality of the analysis and of the predictions. Following specific guidelines can prevent undesired disturbances from light, temperature, and instrument cleanliness. Similar to other portable instruments, SCiO should be calibrated at the

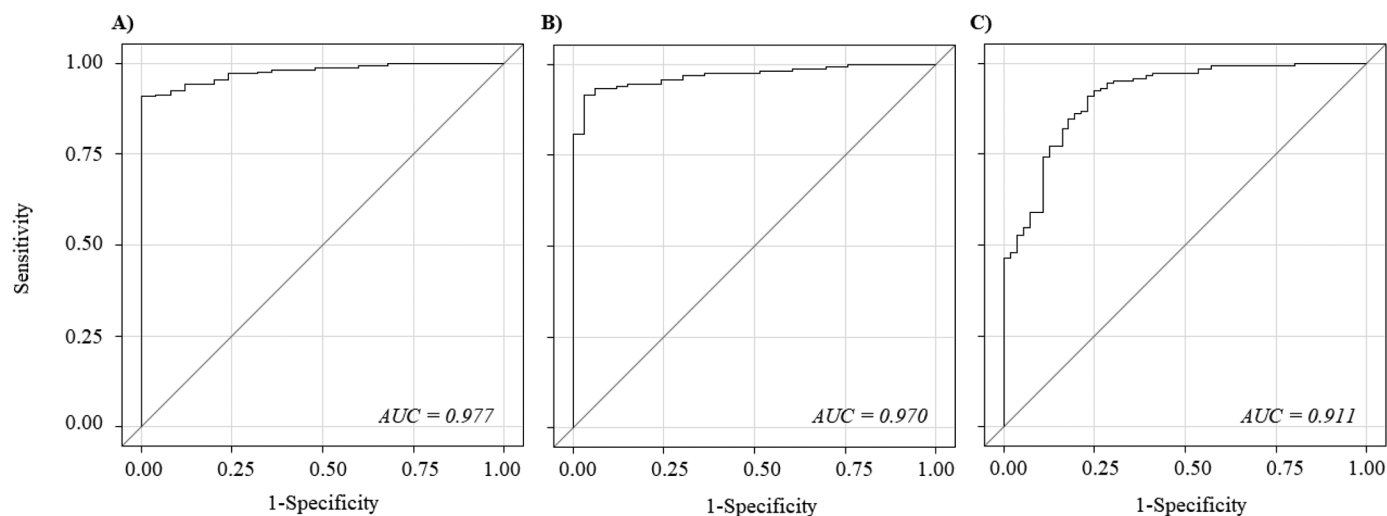


Figure 4. Receiver operating characteristic curves for the IgG concentration predicted by the SCiO spectrometer along with the area under the curve (AUC). Thresholds used for “low-quality” sample classification were (A) <50, (B) <60, and (C) <70 g/L.

beginning of each analysis round—even more than once on a daily basis—as indicated by the manufacturer, by taking a reading on a supplied ceramic white base that serves as a calibration blank sample. Given the portability, promising performance, and cost, such a device opens the door for large-scale studies on BC using data collected in the field by researchers, veterinarians, and farm managers.

CONCLUSIONS

In the present study, we attempted to predict the concentration of Ig, gross composition, minerals, and AA content in BC from spectra recorded using a handheld wireless spectrometer operating in the near-infrared region. Results suggest that this technology has promising prediction performance for certain traits, namely IgG, IgTot, fat, and AA, and excellent accuracy for dry matter, protein, and S in cross-validation. Although the same traits were satisfactorily predicted in external validation, future emphasis could be placed on accounting for increased variability and validating models using samples from different farms. The good performance of the spectrometer for IgG punctual real-time quantification, along with the ability to discriminate between good and low-quality samples, represents an opportunity for dairy farmers. Handheld devices can speed up a large-scale collection of data for targeted purposes, like selective breeding. As an example, a rapid assessment of BC quality can be a useful driver to determine the application and use on farm but also in the food and pharmaceutical industries where BC is used as an ingredient. At the farm level, ensuring that BC of appropriate quality is administered to calves guarantees an optimal passive transfer of immunity.

NOTES

The authors acknowledge Giulia Nardino and Laura Posenato (University of Padova, Padova, Italy) for the technical support during sampling, the personnel of the LaChi laboratory for the chemical analyses, and the 9 farmers who joined the experiment. Ethical approval was not required for the present study per institutional guidelines and local legislation because only farmers interacted with the cows. The animals' owners involved in the study signed a written informed consent, joined the experiment on a voluntary basis, and were associates of the Veneto Region Breeders Association (ARAV, Vicenza, Italy). The authors have not stated any conflicts of interest.

Abbreviations used: Abs = absorbance; AUC = area under the curve; BC = bovine colostrum; D = detrend;

IgTot = total Ig concentration; ISO = International Organization for Standardization; LV = latent variable; MCC = Matthews correlation coefficient; MSC = multiplicative scatter correction; n_{ext} = number of samples used to perform the external validation net of spectral outliers; NIRS = near-infrared spectroscopy; Out = percentage of outliers; R^2_{C} = coefficient of determination of calibration; R^2_{CV} = coefficient of determination of cross-validation; RID = radial-immunodiffusion; RPD = residual predictive deviation; R^2_{V} = coefficient of determination of external validation; Sc-Mt = scatter correction – mathematical treatment; SE_{C} = standard error of calibration; SE_{CV} = standard error of cross-validation; SE_{V} = standard error of external validation; SNV = standard normal variate; SNVD = SNV + detrend; Transfl = transfectance.

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Feasibility of energy dispersive X-ray fluorescence for quantitative prediction of elemental profile in untreated bovine colostrum

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Feasibility of energy dispersive X-ray fluorescence for quantitative prediction of elemental profile in untreated bovine colostrum

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ABSTRACT

Colostrum is the first secretion produced by the mammary gland after calving, providing essential macronutrients and micronutrients to newborns. Monitoring elemental concentrations in bovine colostrum (BC) is crucial, as it serves for the physiological development and health of the neonates. Current gold-standard methods to determine elemental composition of BC are inductively coupled plasma optical-emission spectrometry or inductively coupled plasma MS. These techniques, however, are time-consuming, expensive, labor-intensive, and destructive, and they require sample preparation such as chemical matrix decomposition. In this view, energy dispersive X-ray fluorescence (ED-XRF) could represent a handy alternative. This study evaluated the effectiveness of ED-XRF for the prediction of Na, Mg, P, S, K, Ca, and Zn in BC measured through inductively coupled plasma optical-emission spectrometry as reference method. The performances of ED-XRF in predicting BC elemental composition were assessed on the entire dataset (comprising 160 samples of individual BC), on a training set (comprising 70% of the samples), and on a testing set (comprising 30% of the samples). The models demonstrated satisfactory goodness of fit for S (coefficient of determination in external validation, $R^2_{TE} = 0.77$) and Zn ($R^2_{TE} = 0.74$), indicating their suitability for rough screening of such elements, while showing lower association for Na, Mg, P, K, and Ca. Results of the present study could support the development of rapid screening approaches to assess S and Zn levels in BC, contributing to more informed supplementation strategies of such minerals for calves, thereby preventing deficiencies and supporting neonatal development.

Key words: dairy cattle, correlation, screening, prediction models, health

INTRODUCTION

Bovine colostrum (BC), the first secretion produced by the mammary glands after calving, plays a critical role in providing essential nutrients to newborn calves. Beyond its well-recognized role as a source of antibodies, colostrum is also a vital source of energy, macronutrients, and micronutrients, including key elements (Blum and Hammon, 2000). These elements are essential for the physiological development and health of neonatal calves, particularly for the formation and maintenance of skeletal tissue and support numerous metabolic processes. Major elements such as Na, Mg, Ca, P, S, and K are indispensable for these functions. In addition, trace elements such as Zn contribute to processes such as nutrient transport and muscle function (Shah et al., 2019; Silva et al., 2024).

The composition of BC is specifically tailored to meet immediate nutritional needs of the newborn calf (McGrath et al., 2016). In fact, studies indicate that BC contains higher concentrations of elements compared with mature milk (Abd El-Fattah et al., 2012; McGrath et al., 2016; Playford and Weiser, 2021). However, factors such as dam's diet and parity can influence its mineral composition (Kehoe et al., 2007). As a result, monitoring BC elemental profile could benefit livestock management, enabling dairy farmers to refine calf feeding protocols. This would allow for appropriate mineral supplementation, aiming to prevent deficiencies and to support growth and development of newborn calves. In addition to its role in calf health, the mineral content of BC holds significant value in evaluating the quality and safety of BC-based products entering the human market. Therefore, a deeper understanding of BC elemental composition would provide valuable insights into assessing nutritional value and ensuring the quality and safety of functional foods, supplements, and pharmaceuticals that contain BC (Costa et al., 2023; Goi et al., 2023a). Overall, these considerations highlight the relevance of analyzing colostrum elemental composition for both veterinary and growing industrial applications.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

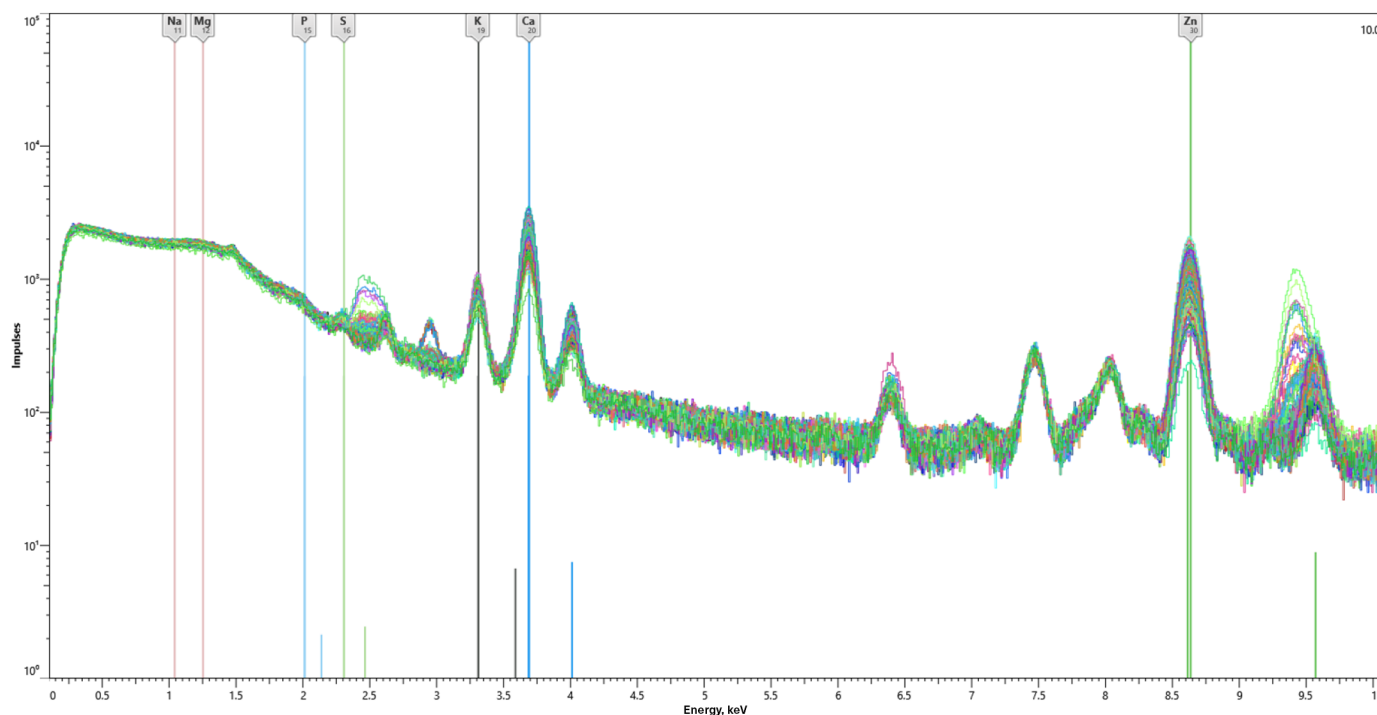


Figure 1. X-ray fluorescence spectra of untreated colostrum samples ($n = 160$) and excitation energies associated with elements included in the study.

Reference methods for element quantification, such as inductively coupled plasma optical-emission spectrometry (**ICP-OES**) and inductively coupled plasma MS, are time-consuming, expensive, labor-intensive, and require extensive sample preparation, such as mineralization procedures (Rawat et al., 2022; Ward, 2024). Recent advancements in analytical techniques, such as energy dispersive X-ray fluorescence (**ED-XRF**), have greatly improved the efficiency and cost effectiveness of element detection and quantification in a wide array of biological substrates (Pashkova et al., 2018). Energy dispersive X-ray fluorescence offers several advantages, including the possibility to avoid the use of toxic reagents (Herreros-Chavez et al., 2019) and bypassing the application of laborious mineralization protocols, making it suitable for samples with high OM content, such as milk (Smagunova and Pashkova, 2013). Recent studies have demonstrated different degrees of goodness of fit in the application of ED-XRF for the prediction of element concentration in dairy products, including milk (Visentin et al., 2023a), cheese (Visentin et al. 2023b), whey powders (Pozza et al., 2024), infant and toddler formulas (Rossmann et al., 2016; Papachristodoulou et al., 2018; Herreros-Chavez et al., 2019), and other milk-based products (Perring and Andrey, 2003).

To the best of the authors' knowledge, there are no studies attempting to apply ED-XRF for the prediction of

elements in BC. This study aims to address the feasibility of ED-XRF in predicting concentrations of Na, Mg, P, S, K, Ca, and Zn in BC.

MATERIALS AND METHODS

Sample Collection

Individual first-milking BC samples were collected from 160 cows of different breeds (84 Simmental, 70 Holstein, 5 crossbred, and 1 Brown) that calved between January 2022 and February 2023, across 40 farms located in Veneto region (Italy), within 6 h after calving. The herds selected for this study originated from commercial farms of similar size (from 90 to 140 cows), feeding systems (based on corn silage and concentrate feeds, distributed as a TMR), and management practices (freestall barns). All cows were reared under an intensive system with no access to grazing pasture but with exercise paddock availability. The average parity was 2.5 ± 1.49 , with 35.0% of cows being primiparous, 25.6% in their second parity, 16.3% in their third parity, and 23.1% with higher parity. Farmers were responsible for collecting colostrum samples in 50-mL plastic tubes and for recording both the cow ID and calving date on the sample tubes, according to the protocol described by Costa et al. (2021). Sampling

occurred exclusively when the dam was promptly separated from the calf, excluding cases of overnight births, dam nursing of the calf, or the dam remaining with the newborn during the initial hours after birth. Immediately after collection, samples were shipped at 4°C to the laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment at the University of Padova (Legnaro, Italy). Upon arrival, each sample was brought to room temperature, homogenized by inversion, and divided into 2 aliquots. One aliquot was designated for reference analysis using ICP-OES, and the other for predictive analysis using ED-XRF. Both aliquots were then stored at -20°C until the respective analyses were performed.

ICP-OES Reference Analysis

One aliquot of BC was thawed, underwent a mineralization process, followed by ICP-OES analysis for element quantification. Sample mineralization was performed according to the protocol described by Franzoi et al. (2018). Briefly, 200 mg of sample were weighed into a Teflon vessel using a Sartorius Entris analytical scale (accuracy ± 0.1 mg, Sartorius Lab Instrument, Göttingen, Germany), followed by the addition of 2 mL of 30% H_2O_2 and 7 mL of 67% HNO_3 . Vessels were hermetically sealed and subjected to microwave digestion using a Milestone Start D system (Milestone Srl, Bergamo, Italy). The digestion program included (1) heating up to 200°C in 15 min, (2) maintaining the temperature at 200°C for 18 min, and (3) cooling to 35°C. Digested samples were diluted to a final volume of 25 mL with ultrapure water produced using a Sartorius Arium 611 UV system (Sartorius, Monza Brianza, Italy).

Elemental concentrations of mineralized samples were determined as described by Franzoi et al. (2018) via ICP-OES using a Ciro Vision EOP system (Spectro Analytical Instruments GmbH, Kleve, Germany) at the following wavelengths: Na, 589.592 nm; Mg, 285.213 nm; P, 177.495 nm; S, 182.034 nm; K, 766.491 nm; Ca, 396.847 nm; and Zn, 213.856 nm. Five-point calibration curves were obtained using external standards (Inorganic Ventures, Christiansburg, VA) diluted in 5% HNO_3 . All calibration curves exhibited a coefficient of determination ≥ 0.99 . The ICP-OES method adopted in the present study was previously validated by Franzoi et al. (2018), who obtained (1) CV in repeatability (i.e., interassay CV) ranging from 0.63% to 4.60% (for K and Ca, respectively), (2) CV in reproducibility (i.e., intra-assay CV) ranging from 2.09% for K to 3.14% (for K and Mg, respectively), and (3) recovery rates ranging from 92% and 97% (for K and Ca, respectively).

ED-XRF Predictive Analysis

The second aliquot of BC was thawed and analyzed without applying any treatment, using a benchtop Spectro Xepos 5P ED-XRF (Ametek, Kleve, Germany) equipped with an X-ray tube anode of Pd and Co (65:35), 50 keV voltage, and 2 mA current. Element prediction was performed based on Na k- α (1.04 keV), Mg k- α (1.25 keV), P k- α (2.01 keV), S k- α (2.31 keV), K k- α (3.31 keV), Ca k- α (3.69 keV), and Zn k- α (8.64 keV). Signal overlap and matrix effects were corrected using the method of influence coefficients (Rousseau, 2006), applied via ED-XRF Spectro Analyzer Pro software (Ametek, Kleve, Germany). For spectra acquisition, 5 g of each sample was weighed on a Sartorius Entris analytical scale (accuracy ± 0.1 mg, Sartorius Lab Instrument, Göttingen, Germany) in disposable ED-XRF plastic cups (32 mm diameter, 24 mm height). To enhance instrument precision, matrix type and exact sample weight were entered into the software. The chamber of the ED-XRF instrument was filled with He to reduce the attenuation of low-energy X-rays. Due to its lower density compared with air, He minimizes scattering and absorption, particularly for lighter elements, thereby enhancing detection sensitivity. The instrument took 4 min to analyze each sample. Energy dispersive X-ray fluorescence spectra obtained from all analyzed samples are shown in Figure 1.

Statistical Analysis

Energy dispersive X-ray fluorescence software reports raw data as energy (keV) versus normalized impulses (counts per second) and converts normalized impulses to predicted concentration of the i th element (Na, Mg, P, S, K, Ca, and Zn) through the Lucas-Tooth formula (Lucas-Tooth and Pyne, 1963) implemented in the XRF analyzer pro software (v. 3.9.3, Ametek, Kleve, Germany). Outliers were defined as residuals deviating by more than 3 SD from the mean. Residuals were calculated as the difference between the reference values and the predicted values (Benedet et al., 2019; Goi et al., 2023b). For each element, outliers were identified separately (1 for Na, 2 for Mg, 3 for P, 2 for S, 2 for K, 3 for Ca, and 2 for Zn), set as missing, and were excluded from subsequent statistical analyses. After outlier removal, performances of ED-XRF in estimating element concentrations were assessed using 3 datasets: (1) a calibration set (100% of the samples), (2) a training set (70% of the samples), and (3) a testing set (30% of the samples). To cover the entire phenotypic variability of the original dataset, samples included in training and testing sets were independently selected for each element.

Energy dispersive X-ray fluorescence calibration was performed after outlier removal on the calibration set

(which included 159 records for Na, 158 for Mg, 157 for P, 158 for S, 158 for K, 157 for Ca, and 158 for Zn), as a regression between the concentrations of the i th element (mg/100 g) measured through ICP-OES reference analysis versus the concentration of the i th element (mg/100 g) predicted through ED-XRF technique, using the Lucas-Tooth formula as previously described. The same regression was iterated for each element, leading to the development of 7 specific equations. The goodness of fit of each prediction was determined through the coefficient of determination in calibration (R^2_C), calculated in accordance with the recommendations of Alexander et al. (2015). The robustness of the results obtained in the calibration was assessed through 2 further trials, namely training and testing.

Energy dispersive X-ray fluorescence training was performed on the training set (which included 106 records for Na, 106 for Mg, 104 for P, 106 for S, 105 for K, 105 for Ca, and 106 for Zn), as a regression between the concentration of the i th element determined through ICP-OES versus the concentration of the i th element predicted through ED-XRF technique, using the Lucas-Tooth formula as previously described. This resulted in 7 training equations, each corresponding to a specific element. The agreement between measured and predicted concentrations was assessed through the coefficient of determination in training (R^2_{TR}).

Energy dispersive X-ray fluorescence validation was performed on the testing set (which included 53 records for Na, 52 for Mg, 53 for P, 52 for S, 53 for K, 52 for Ca, and 52 for Zn entirely separate from those included in the training set), by regressing the concentration of the i th element measured through ICP-OES against the concentration of the i th element predicted by ED-XRF using the previously developed training equation. The agreement between measured and predicted concentrations was evaluated using the coefficient of determination in testing (R^2_{TE}). Recovery was calculated as the percentage ratio between concentrations predicted by ED-XRF and those measured by ICP-OES. This metric was selected to assess model performance, as it provides a direct and interpretable measure of predictive accuracy. Alternative indices such as the ratio of performance to deviation were not considered, because they offer limited additional insight beyond that provided by R^2 (Bellon-Maurel et al., 2010; Minasny and McBratney, 2013).

RESULTS AND DISCUSSION

Descriptive Statistics

Descriptive statistics of elements measured through ICP-OES before the removal of outliers are summarized in Table 1. Among analyzed elements, Ca and P were the

Table 1. Descriptive statistics of elements (mg/100 g) in bovine colostrum before outlier removal ($n = 160$), measured by the inductively coupled plasma optical-emission spectrometry (ICP-OES) reference method

Element	Mean	SD	CV, %	Range
Na	70.80	24.35	34.39	7.57–171.98
Mg	34.74	10.91	31.40	8.68–81.68
P	192.53	51.42	26.71	22.03–323.39
S	162.94	47.42	29.10	11.58–278.71
K	151.18	23.84	15.77	29.17–211.13
Ca	208.20	55.47	26.65	34.53–368.86
Zn	2.07	0.79	38.37	0.27–6.01

most abundant, with average concentrations of 208.20 and 192.53 mg/100 g, respectively. These elements also exhibited the widest concentration ranges, varying from 34.53 to 368.86 mg/100 g for Ca and from 22.03 to 323.39 mg/100 g for P. Sulfur and K presented slightly lower mean concentrations (162.94 and 151.18 mg/100 g, respectively), with comparable concentration ranges. In contrast, Zn and Mg were the least abundant, with concentrations of 2.07 and 34.74 mg/100 g, respectively, with Zn also displaying the greatest variability among all the analytes, reflected by a CV of 38.37%.

The mean Ca concentration in this study (208.20 mg/100 g) was slightly lower than that reported by Goi et al. (2023a; 221.22 mg/100 g), Abd El-Fattah et al. (2012; 281.40 mg/100 g), and Tsioulpas et al. (2007; 217.63 mg/100 g). The average P concentration (192.53 mg/100 g) was comparable to Goi et al. (2023b; 197.82 mg/100 g) but higher than Abd El-Fattah et al. (2012; 53.00 mg/100 g) and Tsioulpas et al. (2007; 92.91 mg/100 g). Zinc concentration (2.07 mg/100 g) was about 10 times higher than that reported by Abd El-Fattah et al. (2012; 0.27 mg/100 g), whereas Mg concentration (34.74 mg/100 g) was slightly lower than that reported by the same authors (42.86 mg/100 g). Kehoe et al. (2007) recorded element concentrations, including S, K, Na, Mg, and Zn, at least 50% higher than those obtained in the present study. Overall, the minimum values of the samples included in the present study were lower than those reported by Goi et al. (2023a) and Kehoe et al. (2007). In contrast, the maximum values were similar to or lower than those reported by Goi et al. (2023b) and Kehoe et al. (2007), respectively. It is important to acknowledge that comparing results with existing literature can be challenging as many studies are based on small sample sizes and different sampling protocols—for instance, in the present study, samples were collected directly by the farmers. These include the adoption of repeated sampling from the same cows or differences in the timing of sampling after calving. Other factors that may have contributed to discrepancies include the proportion of

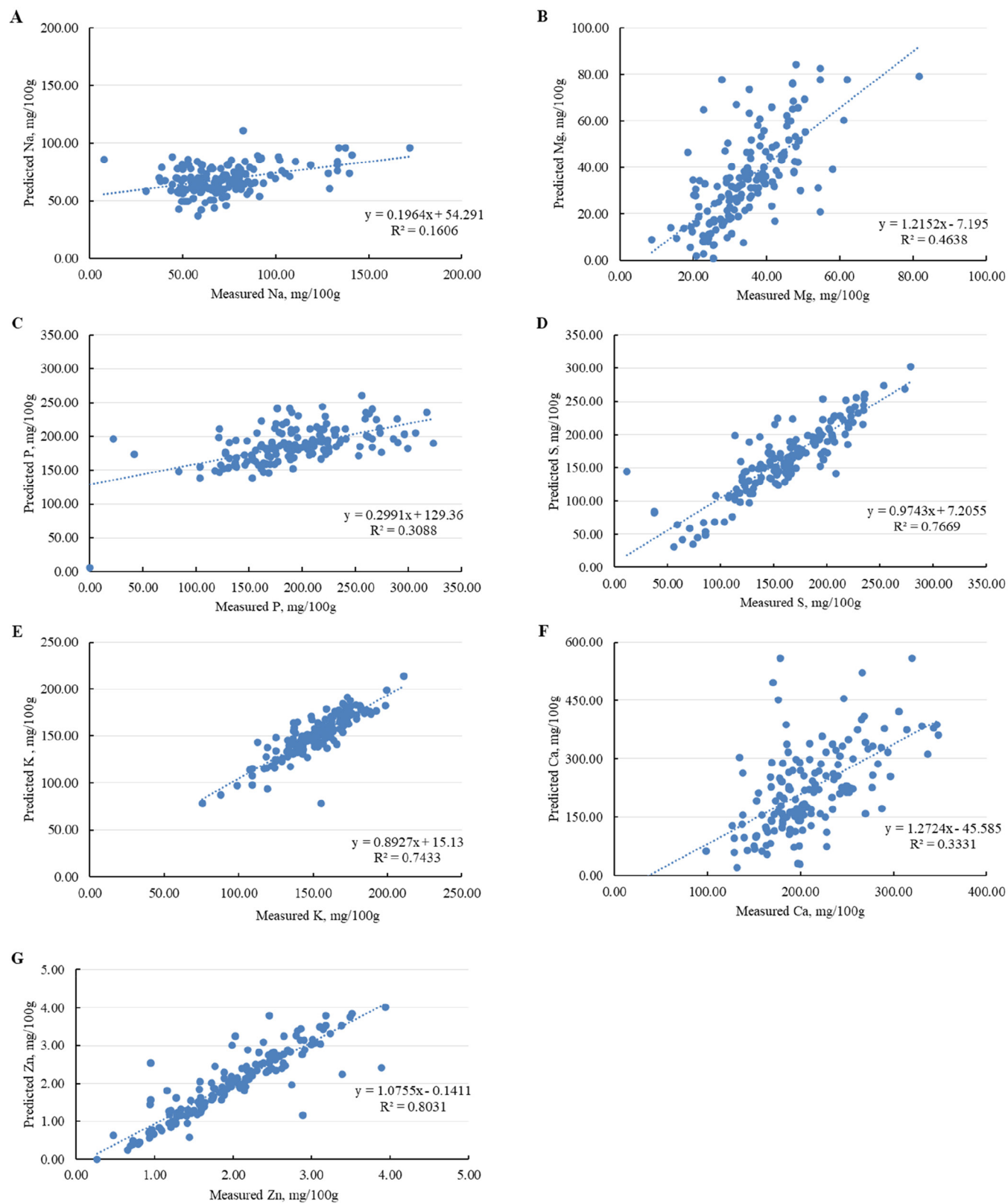


Figure 2. Scatter plots of concentrations (mg/100 g) measured by inductively coupled plasma optical-emission spectrometry (ICP-OES; x-axis) versus concentrations (mg/100 g) predicted by energy dispersive X-ray fluorescence (ED-XRF; y-axis) for Na (A; n = 159), Mg (B; n = 158), P (C; n = 157), S (D; n = 158), K (E; n = 159), Ca (F; n = 157), and Zn (G; n = 158) in the calibration set.

Table 2. Descriptive statistics of elements (mg/100 g) in bovine colostrum samples included in the training and testing set, measured using the inductively coupled plasma optical-emission spectrometry (ICP-OES) reference method

Element	Training set					Testing set				
	n	Mean	SD	CV, %	Range	n	Mean	SD	CV, %	Range
Na	106	70.96	22.62	31.88	30.21–140.94	53	71.27	27.24	38.22	7.57–171.98
Mg	106	34.60	11.18	32.33	8.68–81.68	52	34.57	10.32	29.86	15.39–62.00
P	104	194.97	46.69	23.95	83.92–323.39	53	189.36	55.29	29.20	22.03–317.07
S	106	161.74	47.87	29.60	11.58–278.71	52	161.86	46.54	28.76	37.75–273.01
K	105	152.16	22.06	14.50	75.51–211.13	53	152.23	21.68	14.24	88.08–199.37
Ca	105	207.25	51.11	24.66	36.03–346.50	52	211.43	50.40	23.84	129.07–348.12
Zn	106	1.97	0.76	38.71	0.27–3.94	52	1.97	0.72	36.68	0.66–3.51

parity orders of the cows, where primiparous cows are known for producing colostrum with greater concentration of Ca, P, and Mg compared with multiparous (Silva et al., 2024). Also, the season of calving, breed, and nutritional status of the dam can influence the content of elements in BC (Kume and Tanabe, 1993; Zarcula et al., 2010). Additionally, inherent variability in BC should be considered when interpreting results from different studies (Costa et al., 2023).

Calibration Set

Linear regression plots of element concentrations measured through ICP-OES versus those predicted through ED-XRF in calibration, along with the corresponding R^2_C , are shown in Figure 2. According to the thresholds proposed by Grelet et al. (2021), good prediction performances were achieved for Zn ($R^2_C = 0.80$), S ($R^2_C = 0.77$), and K ($R^2_C = 0.74$), making the models suitable for a rough screening. For the other elements, R^2_C were below 0.47, indicating a poor fit of the model.

Coefficients of determination in calibration in this study were generally higher compared with those reported by Visentin et al. (2023b) who assessed ED-XRF for the prediction of Na, Mg, P, S, K and Ca content in non-lyophilized milk. The same authors reported greater R^2_C when assessing ED-XRF for the prediction of elements in cheese, with the exceptions of Mg ($R^2_C = 0.23$) and S ($R^2_C = 0.70$). Pozza et al. (2024), who applied ED-XRF for the prediction of elements in skim milk and whey powders, reported higher R^2_C for Na (0.40), P (0.93), K (0.88), Ca (0.97), and Zn (0.99), even if the goodness of fit of their predictions may have been affected by the relatively small sample size ($n = 23$). The same authors reported that elements with a greater molar mass such as Ca (40.07 g/mol) and Zn (65.38 g/mol), exhibited the greatest R^2 among the studied elements, whereas those with a lower molar mass, such as Na (22.9 g/mol) and Mg (24.31 g/mol), exhibited the lowest R^2 . Their results are comparable to those observed in this study where

elements with a relatively great molar mass and excitation energies, such as Zn (65.38 g/mol; 8.63 keV) and K (39.09 g/mol; 2.01 keV), exhibited high and distinct peaks (Figure 1), resulting in accurate predictions. However, S denoted a high prediction correlation with the second-best R^2_C value (0.77), despite presenting high background noise similarly to lighter elements, namely Na, Mg, and P. In contrast, Ca (40.07 g/mol), with a molar mass comprised between those of K (39.09 g/mol) and Zn (65.38 g/mol) and a well-defined peak, displayed a low R^2_C value (0.33).

Overall, results suggest that BC, likely due to its higher water content, is more susceptible to interferences compared with cheese, skim milk powders, and whey powders. This is consistent with findings that ED-XRF tends to be more accurate in predicting element concentrations in matrices with relatively low water content (Byers et al., 2019).

Training and Testing Sets

Descriptive statistics of elements measured through ICP-OES for samples included in training and testing sets are reported in Table 2. The greatest differences between training and testing sets were observed for P and Ca concentrations (+2.88% and −2.00% in training, respectively). Despite these differences, P and Ca exhibited some of the lowest CV values in both training set (23.95% and 24.66%, respectively) and testing set (29.20% and 23.84%, respectively). Even smaller differences were observed for other elements, with Zn having the same average concentrations in training and testing sets. Zinc presented the greatest CV in the training set (38.71%) and the second highest in the testing set (36.68%). Potassium presented the second lowest difference in means (−0.05%) in the training set compared with the testing set, along with the lowest CV in both training and testing sets (14.50% and 14.24%, respectively). Overall, these data prove that each element was well represented in both the training and testing sets,

Table 3. Fitting statistics¹ expressing the goodness of fit between data measured through inductively coupled plasma optical-emission spectrometry (ICP-OES) and data predicted through energy dispersive X-ray fluorescence (ED-XRF) within the training and testing sets

Element	Training set		Testing set		
	R ² _{TR}	SE _{TR}	R ² _{TE}	SE _{TE}	Recovery, %
Na	0.24	10.14	0.08	12.40	122.45
Mg	0.49	8.04	0.41	7.79	103.66
P	0.30	39.50	0.14	51.57	119.78
S	0.77	23.11	0.77	22.56	103.03
K	0.79	10.24	0.68	12.37	98.10
Ca	0.36	39.04	0.30	42.50	98.85
Zn	0.83	0.31	0.74	0.37	100.23

¹R²_{TR} = coefficient of determination in training set; SE_{TR} = standard error in training set; R²_{TE} = coefficient of determination in testing set; SE_{TE} = standard error in testing set.

with comparable concentrations and variability, as an essential condition to properly assess the effectiveness of any predictive method (Guerra et al., 2024).

Fitting statistics calculated for training and testing sets are summarized in Table 3, and scatter plots of ICP-OES measured concentrations versus ED-XRF predicted concentrations in testing set are shown in Figure 3. In the training set, the greatest correlations were achieved for Zn (R²_{TR} = 0.83), K (R²_{TR} = 0.79), and S (R²_{TR} = 0.77). Lower accuracies were observed for Mg (R²_{TR} = 0.49) and Ca (R²_{TR} = 0.36), with even lower values for P (R²_{TR} = 0.30) and Na (R²_{TR} = 0.24). The R²_{TR} values obtained in the present study generally exceed those reported by Visentin et al. (2023b), who achieved a maximum R² in calibration of 0.45 for S in their investigation on the prediction of element concentration in nonlyophilized milk using ED-XRF.

Prediction performances on testing set were consistent with those obtained from the training set except for S, which became the best-predicted element (R²_{TR} = 0.77), surpassing Zn (R²_{TR} = 0.74) and K (R²_{TR} = 0.68). Descriptive statistics of concentrations determined by ED-XRF are provided as Supplemental Table S1 (see Notes). Coefficient of determination in testing were generally lower than those obtained in the training, except for S, for which the R² value remained constant across the 2 trials. Goi et al. (2023b), who investigated the ability of mid- (MIR) and near-infrared spectroscopy (NIR) for the prediction of elements in BC, observed higher correlations values in external validation for Ca (R² = 0.66 using MIR and 0.49 using NIR), P (R² = 0.80 using MIR and 0.52 using NIR), Na (R² = 0.53 using MIR and 0.08 using MIR). As the study from Grelet et al. (2021), does not provide a classification for model performance in testing set, recovery values were examined alongside R²_{TE} to assess prediction accuracy (Table 3). For each element, recovery was calculated as the percentage ratio between

concentrations predicted through ED-XRF over concentrations measured through ICP-OES. In the present study, S and Zn, which exhibited the highest R²_{TE} values (0.77 and 0.74, respectively), also showed recoveries close to 100% (103.03% and 100.23% respectively). The results suggest that the predictive model slightly overestimates the concentration of S in BC. Overall, the findings confirm the model's acceptable predictive performance for both S and Zn. Conversely, elements with lower R²_{TE}, exhibited lower or higher recoveries, suggesting a consistent underestimation or overestimation of predicted element concentrations.

Further studies involving untreated BC from diverse populations, or BC subjected to pretreatments to reduce water content (e.g., dehydration or palletization), are required to refine the models and attempt to achieve more accurate predictions of the main elements. Despite this, it is worth noting that any sample preliminary treatment would reduce the advantages that ED-XRF technology offers, particularly in terms of time, labor, and simplicity of the analysis. Indeed, an effective application of ED-XRF prediction models for the prediction of element concentrations in BC could significantly aid in ensuring adequate supplementation in calves, preventing deficiencies, and supporting neonatal development. Furthermore, efficient prediction of element content in pooled BC could be beneficial in both farm management and in the production of foods and products intended for human consumption. Although the models developed in the present study showed potential, their performance is not comparable to that of ICP-OES and may be further enhanced with larger, more diverse datasets or the inclusion of additional calibration factors. The insufficient goodness of fit for other elements may be attributed to the high water content in BC, which lead to high background noise and a reduced signal-to-noise ratio, which ultimately makes the prediction of elements less effective (Byers et al., 2019). As shown in Figure 1, Na and Mg, being the elements with the lowest molar mass and the lowest excitation energies among those studied, exhibited high background noise. Sodium and Mg also displayed the lowest average concentration among all elements considered in the present study (Tables 1 and 2), which may have contributed to exacerbating difficulties in their prediction. Similarly, P exhibited high background noise and lacked distinct peaks (Figure 1), indicating its susceptibility to interferences. In contrast, S, which is slightly heavier than P (32.06 and 30.97 g/mol, respectively), showed the highest prediction correlation, despite the high background noise (Figure 1). Zinc, which was the second-best-predicted element, also displayed distinct high peaks. Overall, these results confirm that ED-XRF is more effective in predicting elements with higher molar mass and excitation energies.

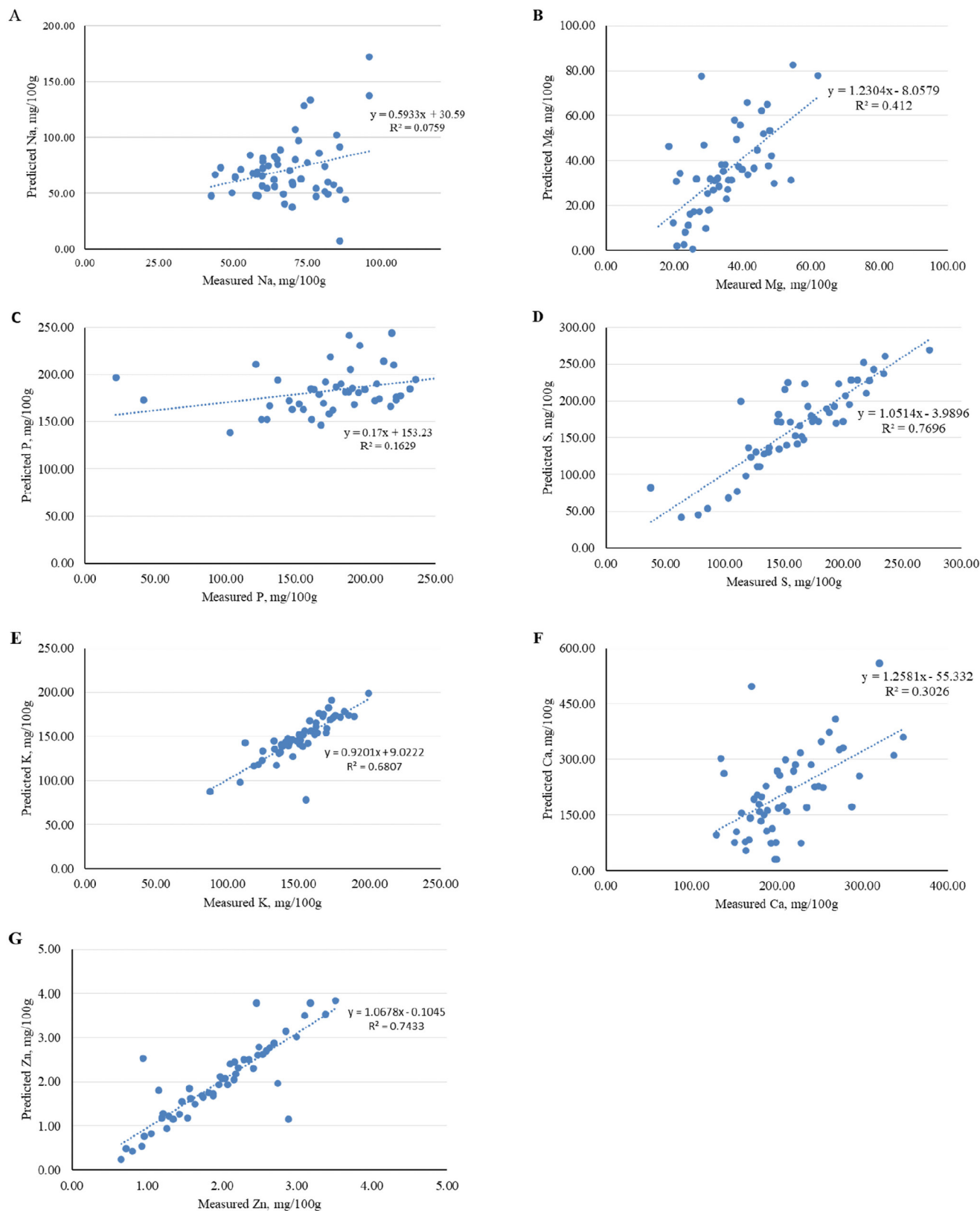


Figure 3. Scatter plots of concentrations (mg/100 g) measured by inductively coupled plasma optical-emission spectrometry (ICP-OES; x-axis) versus concentrations (mg/100 g) predicted by energy dispersive X-ray fluorescence (ED-XRF; y-axis) for Na (A; n = 53), Mg (B; n = 52), P (C; n = 53), S (D; n = 52), K (E; n = 53), Ca (F; n = 52), and Zn (G; n = 52) in the testing set.

CONCLUSIONS

This study assessed the capability of ED-XRF for the quantification of Na, Mg, P, S, K, Ca, and Zn in BC. Among these, S and Zn, showed the strongest predictive performance and among the best values in terms of recovery, making them suitable for screening applications. However, the models developed for other elements did not demonstrate adequate performance in terms of both R^2 and recovery for practical application. Further studies on untreated or pretreated BC are needed to improve prediction accuracy. However, it is worth noting that any pretreatment may reduce the key advantages of ED-XRF, such as speed, labor efficiency, and simplicity.

NOTES

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Nonstandard abbreviations used: BC = bovine colostrum; ED-XRF = energy dispersive X-ray fluorescence; ICP-OES = inductively coupled plasma optical-emission spectrometry; MIR = mid-infrared spectroscopy; NIR = near-infrared spectroscopy; R^2_C = coefficient of determination in calibration; R^2_{TE} = coefficient of determination in testing; R^2_{TR} = coefficient of determination in training.

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Genetic associations of colostrum yield with antibody concentrations and gross composition for selective breeding in Italian Holsteins

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Genetic associations of colostrum yield with antibody concentrations and gross composition for selective breeding in Italian Holsteins

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ABSTRACT

The quality of bovine colostrum (BC), primarily determined by the concentration of IgG, is essential for the transfer of passive immunity and the development of the gastrointestinal tract in neonates. High IgG concentration in BC is pivotal for the calf at the first meal, but neonates often refuse to voluntarily drink the recommended amount in the first hours of life, and dams frequently fail to produce a sufficient volume of colostrum at first milking. This study seeks to estimate the h^2 of colostrum yield (CY) and its genetic correlations with total Ig and IgG, protein, and fat concentrations for the first time in the Italian Holstein population. Colostral Ig concentration and gross composition were assessed on individual samples collected using ad hoc near-infrared spectroscopy prediction models. The infrared-predicted traits, along with the measured CY, were used as phenotypes ($n = 2,938$) for the estimation of genetic parameters through linear animal models. Parity, photoperiod at calving, and classes of CY significantly altered colostrum composition, with parity having the most important influence. Colostrum of older cows, in fact, had greater total Ig, IgG, and protein concentrations than colostrum of younger cows. Colostral quality and CY were higher when calvings occurred in months with decreasing photoperiod. Also, CY was maximum in second-parity cows. Heritability estimates of IgG and total Ig concentrations were 0.225 ± 0.05 and 0.211 ± 0.05 , approximately twice the h^2 estimate for CY (0.101 ± 0.03). The correlation between CY and IgG concentration was positive (0.219 ± 0.032) at the phenotypic level and negative at the genetic level (-0.147 ± 0.190). Spearman rank correlations between EBV of the colostrum traits and official EBV and indexes, for which selection is ongoing in the Ital-

ian Holstein breed, were generally weak or moderate and not always significant. Specifically, CY was unfavorably associated with the PFT (production, functionality, and type; -0.300) and the IES (economic health; -0.335) indexes, and IgG concentration was favorably linked to the official indexes for udder health/mastitis resistance (0.154) and fertility (0.166), mirroring those calculated for total Ig. Selection toward an intermediate optimum for CY at calving can be potentially pursued in the Italian Holstein breed through a balanced index without incurring additional costs for IgG concentration.

Key words: calf health, transfer of passive immunity, dairy cattle, immunoglobulin

INTRODUCTION

The quality of bovine colostrum (BC), traditionally based on IgG concentration, is of paramount importance for the transfer of passive immunity to the newborn calf. In addition, timely administration of good-quality BC contributes to the development of the gastrointestinal tract of the calf (Westhoff et al., 2024). The internationally accepted threshold for BC quality assessment is 50 g IgG/L (Godden, 2008), which is considered as indicative of sufficient quality if at least 4 L of BC are consumed by the calf in the first hours of life (Costa et al., 2023). If the calf is not able to absorb enough IgG, the transfer of passive immunity is insufficient, increasing risks of calf morbidity and mortality (Costa et al., 2021a).

It is therefore crucial to ensure a proper supply of IgG to neonates by providing an adequate amount of BC at the first meal. High-quality BC allows the farmer to deliver the same amount (g) of IgG at the first meal by providing a lower volume of BC. In other words, when IgG concentration is high, it may not be necessary to supply 4 L of BC as traditionally recommended in the field. On average, a dairy cow yields 7 L of BC at first milking, although this can vary widely, from 2 to 23 L (Zarei et al., 2017).

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Table 1. Summary of fitting statistics¹ of the NIR prediction models developed for the colostral concentrations of IgG, total Ig, protein, and fat

Trait	n	Out	SC	MT	LV	SE _{CV}	R ² _{CV}	SE _P	R ² _V
IgG, g/L	529	3.82	SNV	1,8,8,1	14	12.30	0.87	16.80	0.82
Total Ig, g/L	459	3.77	SNV	1,8,8,1	14	13.00	0.87	15.91	0.84
Protein, %	555	5.29	None	1,8,8,1	12	0.60	0.97	1.05	0.93
Fat, %	553	5.47	None	0,0,1,1	14	1.20	0.86	1.82	0.68

¹n = number of samples used to perform the calibration after elimination of outliers; Out = percentage of outliers in the calibration set; SC = scatter correction; MT = mathematical treatment, including derivate order, gap (nm), first smoothing interval, and second smoothing interval (nm); LV = latent variables; SE_{CV} = standard error of cross-validation; R²_{CV} = coefficient of determination of cross-validation; SE_P = standard error of prediction; R²_V = coefficient of determination of external validation.

Ideally, a farmer would prefer cows that produce highly concentrated BC, to avoid administration of excessive volumes to neonates, which frequently refuse to drink 4 L at the first meal. However, dairy cows may fail to produce enough BC at first milking (Costa et al., 2023; Rossi et al., 2023). Rossi et al. (2023) observed that only 60% of Holstein cows produce enough colostrum for the first 2 meals administered to calves, and it has been reported that approximately 21% to 29.1% of cows produce colostrum of poor quality (Bartier et al., 2015; Westhoff et al. 2023). Both colostrum yield (CY) and quality are affected by factors such as season of calving, parity, exposure to pathogens, milk production level of previous lactation, metabolic health, and breed, as well as diet, prepartum management, calf birth weight, gestation length, calving ease, and length of previous lactation (Maunsell, 2014; Immler et al., 2021; Borchardt et al., 2022; Westhoff et al., 2024).

Based on the existing literature, it is not clear whether a genetic relationship exists between CY and the narrow-sense BC quality, defined as Ig concentration. Moreover, the genetic determinism of CY remains largely unexplored. Only Soufleri et al. (2019) have estimated the genetic correlation between CY and some BC quality indicators, such as the refractive index of BC (total solid content) and the infrared-predicted protein concentration. Therefore, the aim of this study was to estimate h^2 of CY and its genetic correlations with concentrations of total immunoglobulin (Ig_{TOT}), IgG, protein, and fat.

MATERIALS AND METHODS

Preliminary Data Cleaning

Data from the first milking of Holstein cows (n = 3,530) that calved between May 2022 and March 2023 in 90 farms as part of the “ColoXInf” project coordinated by the Breeders Association of the Veneto Region (ARAV, Vicenza, Italy) were initially available to accomplish the aim of the present study. All the cows in the database were clinically healthy at sampling and were selected

randomly based on the farms’ calving plan. The BC sampling protocol of Costa et al. (2021a,b) was adapted to this study. In particular, at the first milking within 6 h from calving, the CY (L) was measured using a graduated bucket, and a single representative BC sample was collected using sterile tubes (120 mL) provided to the farmers in advance. Once filled, the tubes were labeled and stored in the farm freezers at −20°C. In addition to CY, information on cow identification number, calving date, calving time, and sampling time was registered. Based on the protocol, cows had to be milked out (empty milking) to have comparable data and allow for the total removal of the BC present in the mammary gland environment. Farmers were instructed to collect samples correctly and to register the CY only when the dam was immediately separated from the calf, therefore excluding overnight births, suckling cows, and cows that remained with neonatal calves for the first hours of life. Quickness in the collection of samples was required to catch the effective IgG concentration at calving and thereby avoid problems caused by the progressive reduction in antibody concentrations after parturition (Godden, 2008; Conneely et al., 2013).

Out of the 3,530 samples collected in the experimental period, 3,193 were retained for further investigation (71 farms). In particular, the samples discarded contained blatant errors in the recorded CY or missing or unreadable information such as parity, dates and time of calving, and cow’s identification number. SAS software v. 9.4 (SAS Institute Inc., Cary, NC) was used for initial data inspection and editing.

Infrared Prediction of Composition Traits

Samples were periodically retrieved from the farms and transported at controlled temperature (−20°C) to the ARAV milk laboratory (Vicenza, Italy) for near-infrared spectroscopy (NIR) scanning and spectra collection. Briefly, after overnight thawing at 4°C, 10 mL of each BC sample was homogenized by gentle inversion of the tube and poured into a specific slurry cup, and the

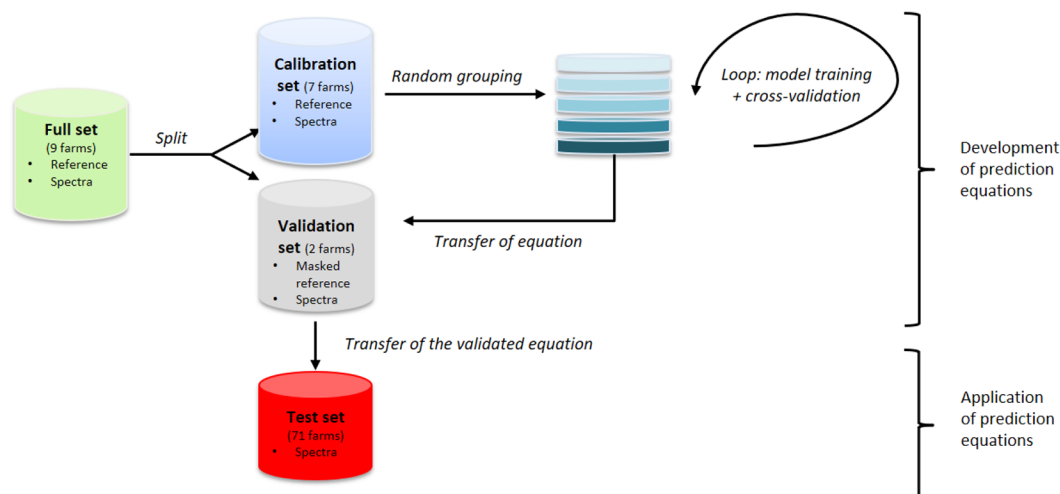


Figure 1. Workflow for the development of NIR prediction models via herd-out external validation (gray cylinder) for colostrum composition traits (97 samples for IgG, 77 for total Ig, 97 for protein content, 97 for fat content) and subsequent application in the external independent test set (red cylinder; 3,193 samples).

spectrum was recorded using the benchtop instrument DS2500 (Foss Electric A/S, Hillerød, Denmark) for prediction purposes.

The prediction models used in this study were developed ad hoc using a pre-existing database comprising samples from 9 farms not included in the external prediction set of the current study for genetic parameter estimation (Figure 1) to avoid overlapping. Samples used for the prediction models included both NIR spectra and reference data determined in the laboratory. The numbers of available reference data for model development ranged from 554 for IgG and Ig_{TOT} to 682 for fat and protein concentrations. Reference data were obtained using the respective gold standards as described in Costa et al. (2021b), with radial immunodiffusion for Ig, the Verbands Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten VI C15.2.1 method (VDLUFA, 2013) for fat, and the Kjeldahl method 991.20 (AOAC, 2000) for protein. Reference values of colostrum IgG, Ig_{TOT}, fat, and protein concentrations averaged 92.48 ± 36.19 g/L, 103.20 ± 36.65 g/L, $4.80 \pm 3.35\%$, and $14.48 \pm 3.74\%$, respectively. Absorbance spectral data recorded by Costa et al. (2021b) were obtained using the same instrument as in the current study, specifically the same infrared interval (400 to 2,500 nm) and the same number of wavelengths ($n = 4,200$). Specific prediction models for IgG, Ig_{TOT}, protein, and fat concentration were developed from this external data set. The herd-out external validation was chosen, with 7 out of the 9 farms in the calibration set and 2 in the validation set (Figure 1). This external validation technique is more realistic than other methods, mimicking the real application of the models in the field. A k-fold cross-validation was performed using

the calibration set and 5 random groups. Prediction models were developed using the WinISI software v. 4.10 (Foss Analytical A/S) and a modified partial least squares algorithm. Detrending, standard normal variate, their combination, and multiplicative scatter correction were tested on spectra in combination with derivative and smoothing parameters 0,0,1,1; 1,4,4,1; 1,8,8,1; 2,5,5,1. In these configurations, the first digit was the derivative, the second digit was the gap over which the derivative was calculated, the third digit was the gap for the first smoothing, and the fourth was the gap for the second smoothing. The number of latent variables (LV) selected for the optimal equation for each trait was the smallest one with the lowest root mean squared error in cross-validation among all the tested LV. Three rounds of outlier elimination were performed, using the following criteria: global $H > 10$, t -statistics > 3 , and X -statistics > 10 . After this step, the standard error in cross-validation (SE_{CV}) and the coefficient of determination in cross-validation (R_{CV}^2), both indicators of the models' performance, were calculated. Each prediction model was finally tested on the validation set ($n = 98$), and the standard error in prediction (SE_P) and the coefficient of determination in external validation (R_V^2) were calculated. After validation, the models, whose performance is given in Table 1, were used to predict IgG, Ig_{TOT}, fat, and protein concentrations in the independent test set of 3,193 samples that contained only spectra but had no reference values available.

For the statistical analysis, the eligible samples were those with the predicted composition traits not exceeding 3 SD from their respective mean. An additional restric-

Table 2. Descriptive statistics¹ of colostrum yield and concentrations of IgG, total Ig, protein, and fat

Trait	Mean	First quartile	Median	Third quartile	Range	CV, %
Yield, L	4.63	3.00	4.00	6.00	0.10–15.00	48.88
IgG, g/L	101.01	78.93	98.44	122.22	0.30–208.59	32.93
Total Ig, g/L	114.13	89.40	111.42	137.88	5.72–242.80	32.28
Protein, %	15.20	13.11	15.16	17.51	2.10–28.19	22.48
Fat, %	4.92	2.69	4.39	6.76	0–22.13	62.01

¹2,938 samples with complete records available; no missing values.

tion was implemented to retain farms with at least 3 cows sampled during the experimental period (May 2022 to March 2023). After these restrictions, the final number of samples (equal to number of cows) was 2,938, and the final number of herds was 60. Cows ranged from parities 1 to 9, with an average of 2.59 ± 1.45 lactations.

Pedigree data were provided by the Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana (ANAFIBJ, Cremona, Italy) and consisted of 19,699 individuals, which included cows with phenotypes and 4 generations of their ancestors.

Genetic and Nongenetic Variation

Univariate analyses were carried out to estimate LSM and genetic parameters for IgG, Ig_{TOT}, protein, and fat concentrations in the ASReml v.4.1 software (Gilmour et al., 2015). The LSM of the BC composition traits were obtained for the fixed effects of parity, photoperiod at calving, and productive level via inclusion of classes of CY. Variance components were estimated for the random effects of herd, additive genetic animal, and residual, according to the following linear animal model:

$$y_{ijklm} = \mu + P_i + S_j + C_k + (P \times C)_{ik} + (P \times S)_{ij} + h_l + a_m + e_{ijklm}, \quad [1]$$

where y_{ijklm} was the phenotypic observation of IgG, Ig_{TOT}, protein, or fat concentration; μ was the overall intercept of the model; P_i was the fixed effect of the i th parity of the cow (from 1 to ≥ 5 , with class 5 including animals from parities 5 to 9); S_j was the fixed effect of the j th photoperiod at calving (2 classes, based on the photoperiod at the latitude where animals were reared: increasing photoperiod and decreasing photoperiod); C_k was the fixed effect of the k th class of CY defined by quartiles (class A: CY ≤ 3.00 L; class B: $3.01 < \text{CY} \leq 4.00$ L; class C: $4.01 < \text{CY} \leq 6.00$ L; class D: CY ≥ 6.01 L); $(P \times C)_{ik}$ was the fixed interaction effect between parity and CY class; $(P \times S)_{ij}$ was the fixed interaction effect between parity and photoperiod; h_l was the random effect of the l th herd ($l = 1$ to 60) $\sim N(0, \mathbf{I}\sigma_h^2)$, where σ_h^2 was the herd variance and $\mathbf{I}$ an identity matrix of ap-

propriate order; a_m was the random effect of the additive genetic animal $\sim N(0, \mathbf{A}\sigma_a^2)$, where σ_a^2 was the additive genetic variance and $\mathbf{A}$ was the pedigree-based relationship matrix; and e_{ijklm} was the random residual $\sim N(0, \mathbf{I}\sigma_e^2)$, where σ_e^2 was the residual variance. The inclusion of the photoperiod at calving in the model was justified by its known effect on colostrogenesis and thus CY (Gavin et al., 2018; Costa et al., 2023). The multiple comparison of LSM was performed based on the t -statistics and the estimated SE ($P < 0.05$).

Genetic and nongenetic factors affecting CY were investigated using the same linear animal model adopted for BC composition traits [1], but with the exclusion of the productive level:

$$y_{ijkl} = \mu + P_i + S_j + (P \times S)_{ij} + h_k + a_l + e_{ijkl}. \quad [2]$$

Variance components of BC composition traits and CY from univariate analyses were used to estimate the h^2 and the herd contribution to the phenotypic variance ($\%_{\text{herd}}$), using the following formulas:

$$h^2 = \frac{\sigma_a^2}{\sigma_h^2 + \sigma_a^2 + \sigma_e^2}$$

and

$$\%_{\text{herd}} = 100 \times \left(\frac{\sigma_h^2}{\sigma_h^2 + \sigma_a^2 + \sigma_e^2} \right).$$

For each univariate analysis, the significance of adding the additive genetic effect to the model was evaluated using the P -value of each h^2 , obtained via the log-likelihood ratio test (Wilson et al., 2010), with significance threshold set at $P < 0.05$.

Additive genetic and phenotypic variances and covariances of CY with IgG, Ig_{TOT}, protein, and fat concentrations were estimated through separate bivariate analyses. Fixed and random effects included in the linear animal models for CY and BC composition traits were those described previously. The additive genetic and phenotypic

Table 3. *F*-values and significance of fixed effects¹ for the colostrum traits analyzed; percentage of phenotypic variance explained by the herd is given along with the *h*² estimates (SE in parentheses)

Trait	Fixed effect					Herd (SE)	<i>h</i> ² (SE)
	P	S	C	P × C	P × S		
Yield, L	20.11***	11.05***	—	—	0.41	23.04 (3.85)	0.101 (0.03)*
IgG, g/L	92.60***	55.55***	46.58***	2.44**	1.86	9.14 (2.23)	0.225 (0.05)*
Total Ig, g/L	90.95***	50.79***	48.65***	2.45**	1.40	8.89 (2.17)	0.211 (0.05)*
Protein, %	54.08***	9.98**	21.71***	1.97*	1.71	8.99 (2.21)	0.191 (0.05)*
Fat, %	189.11***	3.44	22.00***	0.98	1.74	6.25 (1.70)	0.154 (0.05)*

¹P = parity (821, 754, 675, 375, and 313 records in parities 1, 2, 3, 4, and ≥5, respectively); S = photoperiod at calving (589 records for increasing photoperiod and 2,349 records for decreasing photoperiod); C = colostrum productivity level: 4 classes with 971, 576, 907, and 484 samples, respectively.

P* < 0.05; *P* < 0.01; ****P* < 0.001.

(co)variances were used to calculate the genetic (r_a) and phenotypic (r_p) correlations between CY and BC composition traits as follows:

$$r_a = \frac{cov_{aI,aII}}{\sqrt{\sigma_{aI}^2 \times \sigma_{aII}^2}}$$

and

$$r_p = \frac{cov_{pI,pII}}{\sqrt{\sigma_{pI}^2 \times \sigma_{pII}^2}},$$

where $cov_{aI,aII}$ and $cov_{pI,pII}$ were the additive genetic and phenotypic covariances between traits I and II; σ_{aI}^2 and σ_{aII}^2 were the additive genetic variances of traits I and II; and σ_{pI}^2 and σ_{pII}^2 were the phenotypic variances of traits I and II, respectively.

To evaluate how the inclusion of the productive level (CY class) as fixed effect in the model affected the additive genetic variance of IgG and Ig_{TOT} and their covariance with CY, bivariate analyses were run dropping CY and its interaction with parity as in model [2], so that the cow's yield was not accounted for.

Official Indexes

The EBV of bulls with reliability ≥ 0.30 (*n* = 86, 251, 234, 197, and 132 for CY, IgG, Ig_{TOT}, protein, and fat concentrations, respectively) were used to assess the Spearman rank correlations with the official EBV of the same bulls provided by ANAFIBJ: **PFT** (production, functionality, and type), **IES** (economic health), kilograms of milk, kilograms of protein, MST (udder health), SCC, and fertility indexes (full descriptions available at <https://www.anafi.it/en/genetic-indexes/genetic-evaluation-notes>). Reliability (rel) was calculated for

each animal using the prediction error variance (PEV) of the EBV present in the solution file of the ASReml v.4.1 software and the additive genetic variance of the trait: $r^2 = 1 - (PEV/\sigma_a^2)$.

RESULTS AND DISCUSSION

Descriptive Statistics

Phenotypic variation and genetic parameters of CY were investigated using data from 2,938 calvings, for which the BC sample collected by the farmer was NIR-scanned for prediction of IgG, Ig_{TOT}, and gross composition, and for the subsequent estimation of their phenotypic and genetic association with CY. According to Costa et al. (2021b), the IgG concentration in colostrum of Italian Holsteins predicted by NIR is significantly strongly correlated with that measured using the gold standard radial immunoassay, both genetically (0.854) and phenotypically (0.767).

Descriptive statistics of CY and infrared-predicted composition traits of the 2,938 samples are presented in Table 2. When comparing raw data of primiparous (*n* = 821) and multiparous cows (*n* = 2,117) involved in the present study, the average IgG concentrations were 86.70 ± 28.64 g/L and 106.57 ± 33.29 g/L, respectively (results not shown). A similar trend was observed for Ig_{TOT}, with colostrum of primiparous cows having lower concentration (98.31 ± 31.27 g/L) compared with colostrum of multiparous cows (120.27 ± 37.02 g/L). Protein concentration was lower in primiparous ($14.10 \pm 3.43\%$) than in multiparous cows ($15.62 \pm 3.32\%$), but the opposite was observed for fat concentration, which was, on average, greater in primiparous ($7.25 \pm 3.03\%$) than multiparous cows ($4.02 \pm 2.54\%$).

High IgG concentration in BC would allow the farmer to reduce the volume required by the calf at the first meal. Neonates, in fact, often refuse to voluntarily drink 4 L of BC in the first hours of life (Weaver et al.,

Table 4. Fixed effect of parity for each colostrum trait analyzed, LSM $\pm$ SE

Trait	Parity				
	1	2	3	4	≥ 5
Yield, L	4.24 $\pm$ 0.21 ^c	5.24 $\pm$ 0.21 ^a	5.04 $\pm$ 0.21 ^{ab}	4.89 $\pm$ 0.23 ^{ab}	4.67 $\pm$ 0.24 ^{bc}
IgG, g/L	82.70 $\pm$ 2.70 ^d	93.51 $\pm$ 2.59 ^c	105.85 $\pm$ 2.67 ^b	108.78 $\pm$ 2.94 ^{ab}	115.96 $\pm$ 3.00 ^a
Total Ig, g/L	93.55 $\pm$ 2.99 ^d	105.77 $\pm$ 2.86 ^c	119.08 $\pm$ 2.95 ^b	122.58 $\pm$ 3.25 ^{ab}	130.17 $\pm$ 3.32 ^a
Protein, %	13.83 $\pm$ 0.29 ^d	14.56 $\pm$ 0.28 ^c	15.70 $\pm$ 0.29 ^b	15.98 $\pm$ 0.32 ^{ab}	16.68 $\pm$ 0.32 ^a
Fat, %	7.48 $\pm$ 0.22 ^a	4.04 $\pm$ 0.21 ^b	4.20 $\pm$ 0.22 ^b	4.1 $\pm$ 0.25 ^b	4.35 $\pm$ 0.25 ^b

^{a-d}Different superscript letters within a trait indicate significant differences ($P < 0.05$).

2000; Godden, 2008; Vasseur et al., 2009), and in some breeds, such as Brown Swiss, they are reported to have a low suckling reflex (Meza et al., 2020). Assuming that newborns should ingest at least 200 g of IgG to ensure transfer of passive immunity (Godden et al., 2019), the mean concentrations suggest that 2.42 L and 1.88 L of BC would be necessary when the dam is an heifer or not, respectively. The average IgG and Ig_{TOT} concentrations of this study were lower compared with findings of Rossi et al. (2023) of approximately -15% and -12% , respectively. In fact, Rossi et al. (2023) reported averages of 118.7 and 129.89 g/L for these traits using concentration data obtained via radial immunodiffusion assay. In contrast, the colostral IgG level (41 g/L) reported by Morin et al. (2010) was approximately half those of this study, likely due to a delay in sampling. Average IgG was also slightly greater ($+17\%$) and the range similar to findings of Kessler et al. (2020a), who used a modified ELISA kit for IgG determination and reported values between 12.7 and 204.1 g/L (84.2 g/L on average) in various breeds and crossbred cows milked within 0 to 15 h after calving.

The average protein and fat concentrations were in line with the results of Kessler et al. (2020a), who calculated mean values of $14.04 \pm 3.70\%$ and $5.93 \pm 3.09\%$, respectively. Descriptive statistics of BC gross composition were also calculated for Greek Holsteins by Soufleri et al. (2019), who reported averages of $17.83 \pm 3.97\%$ for protein concentration and $6.37 \pm 3.33\%$ for fat concentration in BC samples collected within 3.9 ± 3.2 h from calving.

Although the range was similar (0.5 to 15.3 L), the average CY (5.5 L) reported by Rossi et al. (2023) in Holstein cows was greater than the average calculated in this study (Table 2). Rossi et al. (2023) measured CY by using a graduated milking bucket within 6 h from parturition. A potential reason could be distortion due to the proportion of primiparous cows involved, which was 28% in the present study but not declared in Rossi et al. (2023).

Various studies have dealt with the BC weight (kg) instead of the volume (e.g., Cabral et al., 2016; Soufleri et al., 2019, 2024; and Westhoff et al., 2023). For example,

the CY at first milking of US Holsteins averaged 7.87 kg and ranged from 0.5 to 40 kg (Cabral et al., 2016). In Greek Holsteins, the average CY was 6.18 kg and varied from 0.5 to 23.5 kg (Soufleri et al., 2019). It is worth noting that the interval between calving and BC first milking may influence the observed CY average and range, and that not all studies sampled BC immediately after calving with no suckling. Compared with this study, in fact, Morin et al. (2010) reported greater CY (7.0 ± 4.9 kg) in Holstein cows milked using a portable milking machine and with a wider collection window extended to almost 1 d postcalving. Results also indicated lower values compared with those described by Soufleri et al. (2019), who recorded greater CY (6.18 ± 3.77 kg) through a portable milking machine within 3.9 ± 3.2 h postpartum. According to Westhoff et al. (2023), cows in parities 1, 2, 3, 4, and ≥ 5 produced on average 4.72, 6.05, 5.87, 5.60, and 5.66 kg of BC, and the percentage of animals with CY < 6.00 kg was higher in primiparous (73.4%) than multiparous cows (26.6%). In addition, Westhoff et al. (2023) observed the same minimum for CY across the parities (0.1 kg) and maximums of 38.6, 29.6, 43.8, 33.1, and 41.8 kg in parity 1, 2, 3, 4, and ≥ 5 , respectively.

Overall, CY varies widely, with CV of 49% in the present study and up to 90% in Cabral et al. (2016), who considered multiparous dairy cows milked 4.75 ± 3.8 h from calving. In accordance with the operational protocol of this experiment, farmers were allowed to collect BC within 6 h from calving. This means that BC sampling could have occurred a few minutes from calving or up to 6 h later, as per protocol indication. Unfortunately, data of sampling time (h) was not available for all the farms; therefore this could not be verified. Apart from this limitation, in all farms milking was performed until complete udder emptying, either at the milking parlor or in the calving box with the milking bucket.

Nongenetic Variation

Composition Traits. Results of model [1] are presented in Table 3. The fixed effects of parity, photoperiod at calving, production level (classes of CY), and the inter-

Table 5. Fixed effects of photoperiod at calving¹ and productivity level² (LSM $\pm$ SE) for each colostrum trait analyzed

Trait	Photoperiod at calving		Productivity level			
	Increasing	Decreasing	≤ 3.00 L	3.01–4.00 L	4.01–6.00 L	≥ 6.01 L
Yield, L	4.96 $\pm$ 0.21 ^a	4.67 $\pm$ 0.19 ^b	—	—	—	—
IgG, g/L	96.18 $\pm$ 2.56 ^b	106.54 $\pm$ 2.26 ^a	111.75 $\pm$ 2.46 ^a	104.63 $\pm$ 2.59 ^b	96.87 $\pm$ 2.43 ^c	92.19 $\pm$ 2.64 ^d
Total Ig, g/L	108.77 $\pm$ 2.83 ^b	119.68 $\pm$ 2.49 ^a	126.07 $\pm$ 2.72 ^a	118.04 $\pm$ 2.86 ^b	109.12 $\pm$ 2.68 ^c	103.69 $\pm$ 2.91 ^d
Protein, %	15.12 $\pm$ 0.27 ^b	15.58 $\pm$ 0.24 ^a	16.13 $\pm$ 0.26 ^a	15.63 $\pm$ 0.26 ^b	15.05 $\pm$ 0.28 ^c	14.59 $\pm$ 0.28 ^d
Fat, %	5.02 $\pm$ 0.21 ^a	4.66 $\pm$ 0.18 ^b	4.23 $\pm$ 0.20 ^c	4.74 $\pm$ 0.20 ^b	5.05 $\pm$ 0.21 ^{ab}	5.36 $\pm$ 0.22 ^a

^{a–d}Different superscript letters within a trait indicate significant differences ($P < 0.05$).

¹The photoperiod on the day of calving was classified as “increasing” if the duration of daylight was equal or greater than the duration of darkness (gradually from winter solstice onward in the temperate zone) and “decreasing” if the duration of daylight was shorter or equal to the duration of darkness (gradually from summer solstice onward in the temperate zone).

²Colostrum yield classes contained 971, 576, 907, and 484 samples, respectively.

action between parity and classes of CY were significant in explaining the variation of IgG, Ig_{TOT}, and protein concentration (Table 3). Parity and CY affected also the fat concentration ($P < 0.001$), whereas photoperiod and interaction between parity and CY did not. Parity had the highest F -value across all models, indicating that this effect had the greatest influence on the variance of the composition traits.

Colostrum composition of heifers is known to be different compared with that of cows in subsequent lactations. In fact, confirming the raw means discussed previously, the lowest LSM for IgG, Ig_{TOT}, and protein concentrations were observed for parity 1 (82.7 $\pm$ 2.7 g/L, 93.55 $\pm$ 2.99 g/L, and 13.83 $\pm$ 0.29%, respectively; Table 4). Based on findings of Dunn et al. (2017) and Cordero-Solòrzano et al. (2022), total protein level and thereby the Ig concentration tended to increase with cow's parity in BC. In fact, older cows produce BC of higher quality (greater density) and, for both IgG and Ig_{TOT}, the greatest LSM were observed in parity ≥ 5 , further confirming that the concentration progressively increased as the parity progressed. However, significant differences were observed only in the first 3 parities (Table 4). The factors responsible for this trend were discussed in the review of Costa et al. (2023), and 2 potential explanations were given. First, it is likely that the greater exposure to pathogens of multiparous than primiparous—and, thus, immune activity of older cows—is the main explanation for the increase of Ig across parities. Second, CY did not increase linearly with parity (Table 4), making the concentration of Ig greater only due to a concentration effect. The LSM of CY obtained for parity 1 in fact was statistically similar to the LSM of parity ≥ 5 .

An inverse trend between photoperiod at calving and Ig concentration, with higher IgG (106.54 $\pm$ 2.26 g/L) and Ig_{TOT} (119.68 $\pm$ 2.49 g/L), was observed when the hours of light decreased (Table 5). Similar conclusions have been reached by other authors, who specifically observed higher IgG concentrations in autumn or win-

ter (Conneely et al., 2013; Dunn et al., 2017). Costa et al. (2021a) observed a peak of IgG concentration in late summer to autumn, which is associated with the decreasing photoperiod in Italy. Moreover, the density of BC could come into play in the case of seasonality. In fact, evidence indicates increased density of BC in autumn in Russia (Strekosov et al., 2008). In the present study a decreasing photoperiod was associated with lower CY (Table 5), suggesting a concentration effect. This agrees with the results of Soufleri et al. (2021), who observed that cows calving during the cold season, with a decreasing photoperiod, produced less compared with the other seasons.

The LSM of protein concentration reasonably followed those estimated for IgG and Ig_{TOT}, as antibodies contribute 80% to the total protein in the first BC (McGrath et al., 2016). On the contrary, the fat concentration decreased, with the maximum LSM estimated for cows in parity 1 (7.48 $\pm$ 0.22%). These findings are in agreement with both Dunn et al. (2017) for dairy cows of various breeds and crossbreeds, and Soufleri et al. (2021) for Holsteins. In addition, protein and fat concentrations in BC had an opposite trend depending on whether calving occurred during the half of the year with either increasing or decreasing hours of light (Table 5). Specifically, days of increasing photoperiod were characterized by high fat and low protein levels, whereas cows calving during days of decreasing photoperiod had opposite values. However, Zarei et al. (2017) did not find any effect of calving season on the BC gross composition.

The trend of LSM of the composition traits across the CY classes is presented in Table 5. Except for fat, the most productive cows had the lowest solids concentration in the BC. In the case of IgG, the LSM was 92.19 $\pm$ 2.64 g/L with a productive level greater than 6 L. This is supported by the negative r_p (-0.219 ± 0.032) estimated between IgG and CY with model [1] and presented in Table 6. Even when the r_p was estimated using model [2], thus excluding the CY class from the fixed effects,

Table 6. Phenotypic (below diagonal) and genetic correlations (above diagonal) of colostrum yield and composition traits estimated; SE in parentheses

Trait	Yield	IgG	Total Ig	Protein	Fat
Yield, L	—	−0.147 (0.197)	−0.156 (0.202)	−0.184 (0.206)	0.017 (0.233)
IgG, g/L	−0.219 (0.032)	—	0.994 (0.003)	0.879 (0.039)	0.093 (0.189)
Total Ig, g/L	−0.214 (0.032)	0.983 (0.001)	—	0.863 (0.043)	0.076 (0.192)
Protein, %	−0.215 (0.032)	0.873 (0.006)	0.873 (0.006)	—	0.325 (0.187)
Fat, %	0.108 (0.031)	−0.099 (0.024)	−0.103 (0.024)	0.065 (0.024)	—

the direction and magnitude of the association remained the same (-0.255 ± 0.031). The LSM indicated that cows producing less (lowest CY class) had at the same time the greatest protein and the lowest fat concentrations. In particular, LSM of protein and fat were $16.13 \pm 0.26\%$ and $4.23 \pm 0.20\%$ with $CY \leq 3.0$ L. Conneely et al. (2013) and Morin et al. (2010) estimated an inverse relationship between BC solids and CY, further confirming the presence of a dilution effect. In highly selected populations such as Italian Holsteins, the dilution effect is evident (Table 5), but in other less productive or dual-purpose cattle breeds, the inverse association between Ig concentration and CY may be weaker or absent. The negative relationship between CY and protein and the positive one between CY and fat concentration were confirmed by the r_p reported in Table 6 (-0.215 ± 0.032 and 0.108 ± 0.031 , respectively). Although Soufleri et al. (2019) found no association between CY and fat content at phenotypic level, based on the literature, it is still unclear why correlations of fat and protein concentrations with CY go in different directions. Certainly, BC is a totally different matrix compared with both transition milk and mature milk; therefore dilution dynamics and solids release pattern in the mammary gland alveolar lumen can be extremely peculiar and not equal. Protein is more abundant than fat in cows' BC, whereas in mature milk generally the concentrations are similar or even opposite. One hypothesis could be that, unlike total protein, which mostly consists of Ig in the first hours after calving, the concentrations of fat and other protein fractions such as caseins are relatively low compared with Ig and likely highly variable from cow to cow. In addition, it cannot be excluded that physiologically there could be a priority in terms of release of solids or nutrients in the first hours after birth, resulting in the Ig fraction being the most affected by dilution. To disclose this divergence and to better explore the dilution effect, further research is recommended, such as by monitoring the fat concentration along with protein hourly after calving.

Yield. Results obtained with model [2] for CY are presented in Table 3. Both parity and photoperiod at calving were significant effects ($P < 0.001$), the former having the greatest influence. It is known that the photoperiod to which the cow is exposed before calving has an influ-

ence on the animal's physiology, including BC synthesis (Costa et al., 2023). The CY, in fact, significantly differed between increasing and decreasing photoperiod (4.96 ± 0.21 vs. 4.67 ± 0.19 L; Table 5). Gavin et al. (2018) reported CY to be low during the last months of the year in Jersey cows farmed in the northern hemisphere and proposed that this could be associated with increased hours of darkness. Some studies have evaluated the influence of the photoperiod on milk yield, and results showed that short-day photoperiods before calving led to greater milk production in the subsequent lactation (Gross, 2022). The underlying endocrine mechanisms involving the role of prolactin in galactopoiesis during bovine lactation have been elucidated recently (e.g., Dahl et al., 2012; Lacasse et al., 2014; Ponchon et al., 2017), and positive effects of daylight exposure have been demonstrated on milk yield (Özçelik et al., 2017). The same applies to CY: the surge in prolactin is affected by photoperiod, having an influence on colostrogenesis as well (Gavin et al., 2018). As regards parity, the lowest CY was estimated for primiparous cows, and the LSM ranged from 4.24 ± 0.21 (parity 1) to 5.24 ± 0.21 L (parity 2; Table 4). The LSM in subsequent parities were lower but only numerically different compared with parity 2: 5.04 ± 0.21 and 4.89 ± 0.23 in the cases of parity 3 and 4, respectively. The low secretion ability of primiparous cows is widely known (Capuco et al., 1997), since the development of the udder is not fully completed when cows reach their first lactation, and the limited alveolar capacity and mammary gland volume can therefore limit the secretion of BC or milk (Lang et al., 2012). The milk production capacity of the mammary gland is largely determined by the abundance of epithelial cells and their degree of differentiation (Boutinaud et al., 2004; Kessler et al., 2020b), emphasizing the influence of developmental constraints on milk yield during the first lactation. In mammals, ductal growth and proliferation of secretory cells increase after the first conception, with further maturation occurring throughout lactation (Knight and Peaker, 1982; Lang et al., 2012).

However, the differences were not statistically significant when comparing parity 2 with parity 3, 4, and ≥ 5 , and, numerically, cows in parity ≥ 5 had CY not far from the CY of primiparous cows (Table 4). This is in line with

the trend observed by Soufleri et al. (2021), who identified primiparous cows as those with the lowest CY and observed statistically similar CY across parities in the pool of multiparous. The CY had the greatest percentage of the phenotypic variance explained by the herd (23%; Table 3) compared with the other traits, indicating that CY is likely influenced by farm management at calving.

Overall Considerations. A limitation of the present study on the exploration of factors affecting CY and compositional BC traits is the absence of data regarding potential presence of risk factors causing deficient CY at first milking or quality such as subclinical mastitis. Moreover, it is not infrequent that cows experience mastitis at or immediately before calving, with a consequent inevitable effect on udder synthesis ability. The study conducted by Maunsell et al. (1998) on 106 Holstein cows explored the effect of subclinical mastitis during colostrogenesis and reported that infected mammary glands are characterized by lower CY and diminished transfer of IgG in the alveolar lumen compared with healthy glands. However, the infection did not alter the concentration of IgG, and thus, according to Maunsell et al. (1998), the rate of passive transfer of antibodies in calves was not affected by subclinical mastitis. In the present study, although only clinically healthy cows were sampled, it cannot be excluded that some cows had ongoing subclinical mastitis or mild inflammation.

Genetic Aspects. The h^2 estimates of IgG and Ig_{TOT} were significant and equal to 0.225 ± 0.05 and 0.211 ± 0.05 , respectively (Table 3). These were the most heritable traits, followed by protein, fat, and CY. The estimate obtained for IgG was in line with the wide range of h^2 reported in the literature, but it is worth highlighting that the phenotypic variance of CY, as well as the additive genetic variance of this study, may differ from those reported by other authors due to important differences in population structure, study design, sampling protocol, unit of measure, time (h) from calving, and milking method (e.g., empty vs. non-empty milking). In southern Ireland, Conneely et al. (2013) reported h^2 of 0.21 ± 0.08 for the CY expressed in kilograms using data from multiple breeds and crossbred animals and, differently from milk, quickness in BC sampling is crucial to capture the true variability of the composition traits, especially IgG. Finally, phenotypic and additive genetic variance can be not representative or quite uncertain and h^2 not significant, especially in studies dealing with smaller data sets. As an example, the h^2 of NIR-predicted IgG concentration (0.09) estimated by Costa et al. (2021b) was smaller than that of the present study and had larger SE (0.147). The same can be said for IgG determined via radial immunodiffusion by Costa et al. (2022; $h^2 = 0.15$) and Altvater-Hughes et al. (2022; $h^2 = 0.04$) in Holsteins. At the same time, the h^2 estimate of the present study

was somewhat lower than that reported by Martin et al. (2021) for IgG determined via radial immunodiffusion (0.28 ± 0.14) in Charolais beef cattle. The h^2 of protein concentration (0.191 ± 0.05) was consistent with that presented by Soufleri et al. (2019) for Greek Holsteins (0.19 ± 0.08), but the h^2 for fat concentration (0.154 ± 0.05) was not, being lower compared with their estimate (0.21 ± 0.08). Overall, CY was less heritable (0.101 ± 0.03) than composition traits, corroborating findings of Soufleri et al. (2019), who demonstrated that CY is less heritable (0.04 ± 0.06) than BC composition, even though the h^2 was not significant (Soufleri et al., 2019).

As discussed by Costa et al. (2021b), the ability to explore the influence of genetics on the BC qualitative aspects is strategic in the era of functional traits, especially considering the recent opinion reports and concerns of the European Food Safety Authority (EFSA AHAW Panel, 2023) and ongoing research in some countries such as Canada (Lynch et al., 2021; van Staaveren et al., 2023). Genetics are important when it comes to disease resistance in calves. Despite the lack of reliable measurements and phenotypes directly recorded on calves, results of the present study suggest that selective breeding to enhance BC quality is feasible. Nevertheless, it is crucial to consider the potential desired or undesired correlations between BC traits and the cow's performance and productivity, which in general should not be compromised. For instance, according to Borchardt et al. (2022), a positive correlation exists between the 305-d milk yield of the previous lactation and the CY, which may influence BC traits when applying selection strategies. Additionally, it is important to note that, at the nucleus level, some animals may be intentionally selected for BC quality and CY to produce dams with better colostrum characteristics. In particular, authors estimated a 0.01% increase in CY for every additional kilogram of milk produced in the previous lactation. A similar conclusion was drawn by Soufleri et al. (2021), who observed that cows with a milk yield above 11,000 kg in the previous lactation produced more CY compared with cows with milk yield below 7,000 kg. It is likely that mammary gland capacity and numbers of functional alveoli are capable of influencing synthesis ability of the udder. Considering the BC quality, Dunn et al. (2017) and Kessler et al. (2020b) showed that IgG concentration was greater in cows with higher yield in the previous lactation. In contrast, according to Kessler et al. (2014), CY at first milking and cumulative milk production over the subsequent lactation showed no significant correlation in both multiparous and primiparous Holstein cows. Thus, their results demonstrated that BC obtained in the first hours after calving is basically unrelated to the entire production level of a cow in subsequent lactation stages. Overall, the findings and considerations suggest

Table 7. Correlations calculated from residual (below diagonal) and herd (above diagonal) covariance estimates of colostrum yield and composition traits; SE in parentheses

Trait	Yield	IgG	Total Ig	Protein	Fat
Yield, L	—	−0.380 (0.146)	−0.381 (0.146)	−0.535 (0.125)	0.167 (0.178)
IgG, g/L	−0.206 (0.044)	—	0.900 (0.009)	0.869 (0.011)	−0.079 (0.191)
Total Ig, g/L	−0.196 (0.044)	0.981 (0.002)	—	0.933 (0.025)	−0.084 (0.191)
Protein, %	−0.154 (0.043)	0.885 (0.039)	0.866 (0.012)	—	−0.048 (0.192)
Fat, %	0.119 (0.042)	−0.150 (0.044)	−0.149 (0.044)	0.018 (0.043)	—

that genetic selection toward milk and BC traits does not appear to be antagonistic.

Table 6 and Table 7 report the correlations between the traits calculated from the covariance components estimated. Except for fat, the r_a between CY and the composition traits were generally negative and similar to the r_p (Table 6), as previously discussed for the LSM in Table 5. When using model [2], without CY class as fixed effect, the r_a between IgG concentration and CY was -0.348 ± 0.234 and the r_p was -0.255 ± 0.031 . In the case of Ig_{TOT}, the r_a and the r_p with CY were -0.378 ± 0.237 and -0.266 ± 0.030 . Apart from the correlations, the estimates of h^2 remained similar to Table 3 when using this model instead of model [1]: 0.222 ± 0.051 and 0.224 ± 0.050 for IgG and 0.215 ± 0.051 and 0.218 ± 0.052 for Ig_{TOT}. In addition, the associations between IgG and Ig_{TOT} were similar to those in Table 4, with r_a and r_p equal to 0.995 ± 0.003 and 0.985 ± 0.001 , respectively. When model [2] was used for both traits in the bivariate analysis, the magnitude of the correlations increased compared with model [1] because part of the variance of IgG and Ig_{TOT} was explained by the fixed effect of productivity level in model [1].

Altwater-Hughes et al. (2022) reported an absence of correlation between CY and IgG concentration in dairy cows, suggesting that factors different than BC volume influenced antibody concentration in BC. Similarly, Soufleri et al. (2019) did not observe r_a between CY and protein concentration, but a negative r_p was reported. In the present study, part of the r_a of CY were negative, -0.184 ± 0.206 with protein, but the strongest was positive and estimated between IgG and Ig_{TOT} concentrations (0.994 ± 0.003 ; Table 6). This r_a was then followed by those calculated between protein and IgG (0.879 ± 0.039) and protein and Ig_{TOT} (0.863 ± 0.043). At both phenotypic and genetic levels, the concentration of fat was very weakly correlated with CY, IgG, and Ig_{TOT} (Table 6). The correlations calculated using the residual and herd covariances (Table 7) generally confirmed the r_a and the r_p . The strongest correlation in Table 7 is the residual one between IgG and Ig_{TOT}, close to unity, whereas the weakest ones were those of fat, for which it can be concluded that nongenetic factors play a role in the determination of nonzero phenotypic and residual correlations with CY (Table 7).

Official Selection Indexes. Even though direct information regarding the previous lactation of multiparous cows in the present study was not available, bulls' EBV were used to investigate potential relationships with Italian Holstein official indexes, including composite indexes such as performance and fitness. The Spearman rank correlations (Table 8) provided evidence that a certain association exists between BC traits and ANAFIBJ official indexes. For example, CY was significantly negatively associated with most of the indexes, including the PFT (-0.300), the IES (-0.335), kilograms of milk (-0.218), and kilograms of protein (-0.310). The genetic architecture of CY is not yet fully understood; nor are the mechanisms that make the associations with official indexes negative. However, the correlations suggested that cows genetically predisposed to produce very high CY at first milking were potentially those with compromised milk-related performance and health during the lactation. It is likely that very high yield at calving is antagonistic with traits of interest. As suggested by various authors for traits such as gestation length (Galluzzo et al., 2024), selection indexes should consider the physiological range of the traits (Table 2), and it may be reasonable that breeders will implement strategies aimed at maintaining the intermediate optimum for CY. This would exclude nonphysiological and extreme situations where CY is either insufficient or too high, and the lack of a strong correlation with antibody concentrations further supports the possibility of optimizing both traits simultaneously.

As regards the composition traits, the correlations were not significant except for fat concentration, which was positively associated with PFT, IES, kilograms of milk, and kilograms of protein, and was negatively associated with the udder health and mastitis resistance index. Additionally, IgG and Ig_{TOT} concentrations showed significant positive association with the udder health and mastitis resistance index and the fertility index.

Soufleri et al. (2024) reported approximate r_a for some colostrum traits, including CY and gross composition predicted via mid-infrared spectroscopy with benchtop instruments used for routine official milk analyses. By using bulls' EBV, those authors observed that the BC protein concentration was negatively correlated with milk yield, productive life length, and livability. Interestingly,

Table 8. Spearman rank correlations calculated between sires' EBV¹ for colostrum traits and some of the Italian Holstein official indexes²

EBV	PFT	IES	Kg milk	Kg protein	SCC	Mastitis	Fertility
Yield, L	−0.300**	−0.335**	−0.218*	−0.310**	0.052	0.216*	−0.134
IgG, g/L	−0.038	0.003	0.014	0.005	0.118	0.154*	0.166**
Total Ig, g/L	−0.027	0.023	−0.005	0.020	0.118	0.162*	0.184**
Protein, %	−0.001	0.024	0.064	0.029	0.075	0.122	0.168*
Fat, %	0.220*	0.210*	0.289***	0.179*	−0.118	−0.225**	−0.009

¹Bulls with reliability ≥ 0.30 were 86, 251, 234, 197, and 132 for colostrum yield, IgG, total Ig, protein concentration, and fat concentration, respectively. The average number of daughters with phenotype per bull was 422 (minimum 3; maximum 215).

²PFT = production, functionality, and type index; IES = health economic index; mastitis = udder health/mastitis resistance index; fertility = fertility index. Full descriptions available at <https://www.anafi.it/media/pubblicazioni/schede-calcolo/Schede-calcolo-Indici-ITA-2024.pdf>.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

the fat concentration of BC and milk were negatively associated with each other. Authors also reported that the colostral lactose concentration was generally positively associated with cows' productive life, livability, and BC protein level and yield. It is, however, important to mention that limited numbers of sires ($n = 61$) and daughters ($n = 854$) were used and that no information was provided regarding accuracy and precision of the mid-infrared spectroscopy models adopted (Soufleri et al., 2024). Direct comparison of the current findings with those of Soufleri et al. (2024) should be done with caution because the Greek and the Italian Holstein populations differ, and no standardized protocol was used to obtain BC phenotypes in the 2 studies. Soufleri et al. (2024) had calf traits available to evaluate if there were sires that could produce offspring with good ability in CY production and neonatal transfer of passive immunity. Considering both top- and bottom-ranking sires, a favorable correlation was observed between BC total solids and total protein in calves' serum, which served as an indicator of passive transfer, both measured with a refractometer (Soufleri et al., 2024). This suggested that some sires are able to generate offspring with favorable and desirable BC characteristics, such as CY and quality at the same time.

CONCLUSIONS

This study highlighted that the CY at first milking is a variable phenotype in Italian Holsteins inversely correlated with antibody concentration. Colostrum yield was lowest in first-parity cows, and it was heritable to a low degree (0.101) compared with IgG (0.225) and Ig_{TOT} concentrations (0.211) predicted via NIR. Estimates make all these traits exploitable for selective breeding toward optimal transfer of passive immunity, which comprises sufficient CY and high IgG at first milking. Optimizing both BC quality and yield at the same time is feasible, but economic weights in selection indexes should be

defined in synergy with all stakeholders to take into account indirect benefit and relevance compared with other traits for which selection is ongoing for a proper balance. Genetic selection for both milk and BC traits appears to be non-antagonistic and can be integrated into breeding programs of Italian Holsteins to improve calf health and survival without compromising economically relevant traits. For this scope, recording calf health data in the field is advisable and pivotal.

NOTES

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Nonstandard abbreviations used: ANAFIBJ = Associazione Nazionale Allevatori della Razza Frisone, Bruna e Jersey Italiana; ARAV = Breeders Association of the Veneto Region; BC = bovine colostrum; CY = colostrum yield; IES = economic health index; Ig_{TOT} = total immunoglobulin; LV = latent variables; MT = mathematical treatment; NIR = near-infrared spectroscopy; PEV = prediction error variance; PFT = production, functionality, and type index; r_a = genetic correlation; r_p = phenotypic correlation; SC = scatter correction; SE_{CV} = standard error of cross validation; SE_p = standard error of prediction.

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General conclusions

Spectroscopic techniques can capture key compositional and immunological features of BC, supporting their use as reliable phenotyping tools and providing clear advantages in terms of speed, scalability, and suitability for large-scale or on-farm implementation. Based on the results obtained, MIR spectroscopy appears to be the most robust and comprehensive approach which can serve as a platform for BC assessment, showing reliability across a wide range of traits. In contrast, Vis-NIR and NIR spectroscopy as well as ED-XRF offered complementary or more targeted benefits, suggesting that these approaches are recommended only when a focused screening is required. Portable spectrometers represent a step toward data-driven colostrum management, aligning with the goals of precision livestock farming and precision feeding, and enabling more efficient colostrum banking practices through rapid on-farm quality classification. Their ease of use and immediate output make them promising tools for informed real time decision-making. Nonetheless, continued refinement of calibration models and the expansion of spectral databases will further strengthen prediction performance.

The present thesis demonstrates that both intrinsic (cow) and extrinsic (environmental) factors can influence the BC quality and yield at first milking, with parity, productivity level, season of calving, and cow genetic merit playing a fundamental role on their variability within the population. Moreover, proper management of BC, including optimizing feeding protocols, can help reduce the risk of PTI failure and improve calf survival and overall farm efficiency. In the longer term, surplus high-quality colostrum could represent an additional resource for alternative applications, including functional foods and products supporting human health. From a genetic perspective, improving BC quality and yield is possible through balanced selection strategies that include both traits in breeding objectives. Combining large-scale spectroscopic phenotyping with genetic evaluation could support selection programs to strengthen passive immunity, calf health, and herd resilience.

List of publications

2023

Costa, A., Sneddon N. W., **Goi A.**, Visentin G., Mammi L. M. E., Savarino E. V., Zingone F., Formigoni A., Penasa M., and De Marchi M. (2023). *Invited review: Bovine colostrum, a promising ingredient for humans and animals - Properties, processing technologies, and uses.* Journal of Dairy Science 106.

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Guerra, A., Simoni M., Longobardi V., **Goi A.**, Mantovani G., Danese T., Neglia G., De Marchi M., and Righi F. (2024). Effectiveness of near-infrared spectroscopy to predict the chemical composition of feces and total-tract apparent nutrients digestibility estimated with undigestible neutral detergent fiber or acid-insoluble ash in lactating buffaloes' feces. Journal of Dairy Science 107:5653–5666.

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National and International Congresses

Oral Presentations

2023

De Marchi, M., **A. Goi**, M. Pozza, E. Chiarin, and A. Costa. (2023). Infrared models for the prediction of cow colostrum immunoglobulins G concentration: phenotypic variability and relationship with colostrum yield. In: Proceedings of the 46th ICAR Annual Conference, 21st – 26th May, Toledo, Spain, vol. 27:233.

Righi, F., Pitino R., Pozza M., **Goi A.**, De Marchi M., and Simoni M. (2023). Relationship between diets characteristics and milk composition in lactating dairy cows fed silage- or hay-based diets. In: Book of Abstract of the 25th ASPA Annual Meeting, 13rd – 16th June, Monopoli, Italy, vol. 22:s1. p. 121.

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Goi A., De Marchi M., Cassandro M., Finocchiaro R., Marusi M., and Costa A. (2024). Colostrum yield is heritable and genetically correlated with immunoglobulins concentration in Holstein cows. In: Book of Abstract of the 75th EAAP Annual Meeting, 1st – 5th September, Florence, Italy, vol. 34:544.

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spectrometer. In: Book of Abstract of the 75th EAAP Annual Meeting, 1st – 5th September, Florence, Italy, vol. 34:313.

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Posters

2023

Goi, A., De Marchi M., Visentin G., Manuelian C.L., and Costa A. (2023). Immunoglobulins concentration and major solids content of bovine colostrum can be accurately determined through mid-infrared spectroscopy. ADSA Annual Meeting, 25th – 28th June, Ottawa, Ontario (Canada).

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2024

Goi A., Costa A., Pozza M., and De Marchi M. (2024). On field application of near-infrared portable spectrometer for cow colostrum quality assessment. ADSA Annual Meeting, 16th – 19th June, West Palm Beach, Florida (US).

Goi A., De Marchi M., and Costa A. (2024). Phenotypic variation of colostrum yield and its association with immunoglobulin G content in Simmental cows. 75th EAAP Annual Meeting, 1st – 5th September 5, Florence (Italy).

Costa A., Visentin G., Masi M., Vecchio Y., **Goi A.**, and De Marchi M. (2024). Colostrum administration quickness and farmer's characteristics affect calves' survival. 75th EAAP Annual Meeting, 1st – 5th September 5, Florence (Italy).

Technical Publications

2024

Goi, A., De Marchi M., and Costa A. (2024). Minerali e aminoacidi essenziali del colostro bovino: fattori che ne influenzano la concentrazione e capacità predittiva della spettroscopia infrarosso. RUMINANTIA. 31st January

Goi A., De Marchi M., and Costa A. (2024). Potenzialità di dispositivi portatili NIR per la predizione della concentrazione di immunoglobuline nel colostro bovino in stalla. RUMINANTIA. 19th August

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