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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 the 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 Landwirdschaftlicher 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 hydrochloride 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-μm filter. The derivatization step of the mixture containing 10 μL 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 μm, 2.1 × 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 ($\mathbf{RMSE_{CV}}$) 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 ($\mathbf{R^2_{CV}}$) and external validation ($\mathbf{R^2_V}$) 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 ith season of calving (December to February, March to May, June to August, and September to November); P_j is the fixed effect of the jth parity (3 classes, where the last included parity orders from 3 to 8); Y_k is the fixed effect of the kth year of calving (2019 and 2020); H_l is the fixed effect of the lth 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 $\sim N\left(0, \sigma_e^2\right)$, 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 $\geq$ 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 statistics[1] 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

[1]Before outlier elimination.

[2]Log_{10}-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 before 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 traits[1] 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

[1]Expressed as mg/100 g, as is, for AA and milligrams per kg, as is, for minerals.
[2]Log_{10}-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 statistics[1] 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

[1]n = 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; R^2_{CV} = coefficient of determination of cross-validation; n_{ext} = number of samples used to perform the external validation; R^2_V = coefficient of determination of external validation; RPD = residual predictive deviation.

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

a better R^2_{CV} for K (0.69). Still in bovine milk, Soyeurt et al. (2009) obtained a greater but still unsatisfactory R^2_{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 statistics[1] 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

[1]n = 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; R^2_{CV} = coefficient of determination of cross-validation; n_{ext} = number of samples used to perform the external validation; R^2_V = coefficient of determination of external validation; RPD = residual predictive deviation.

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

[3]Log_{10}-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

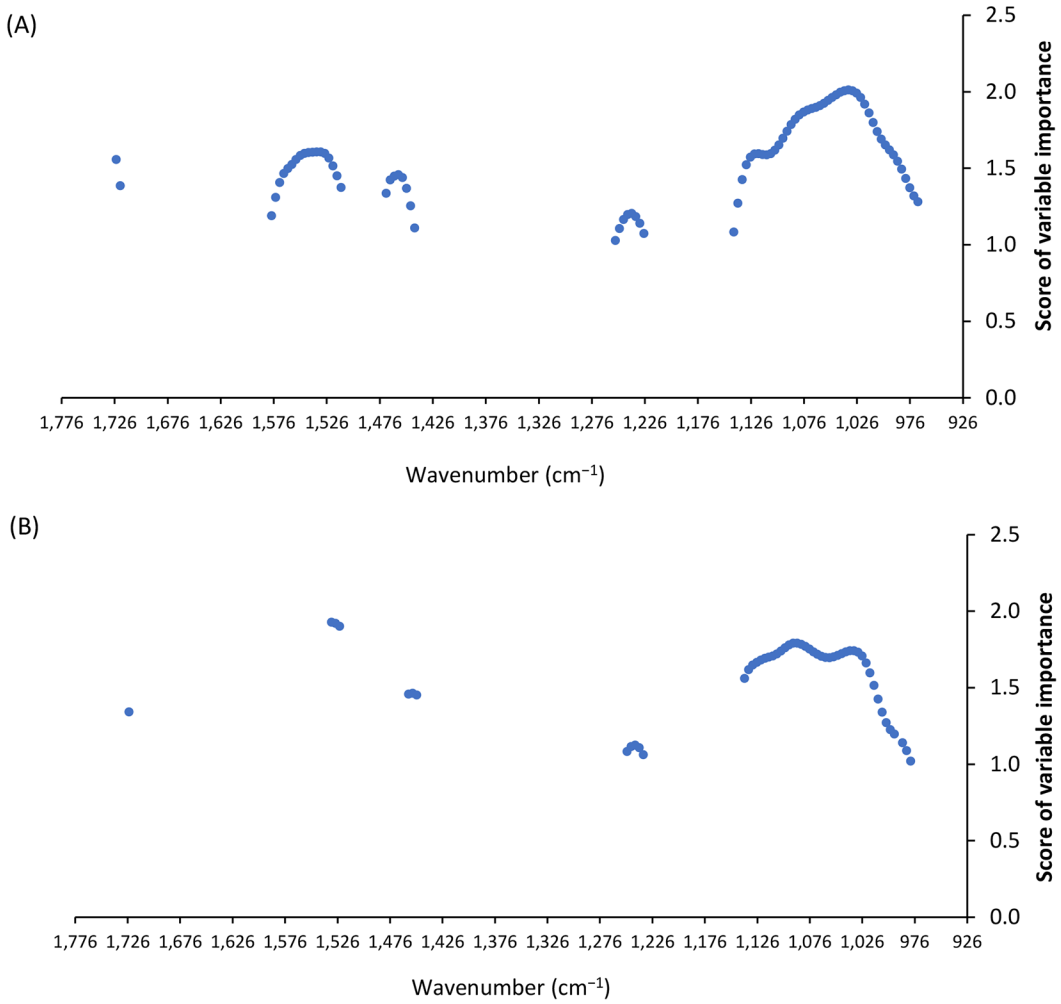


Figure 1. Overview of the most important (variable importance >1; Caponigro et al., 2023) wavenumbers (blue dots) used for prediction of bovine colostrum (A) EAA and (B) mineral concentration via mid-infrared spectroscopy.

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 ± 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/m^3) compared with multiparous cows (1,068 kg/m^3; 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

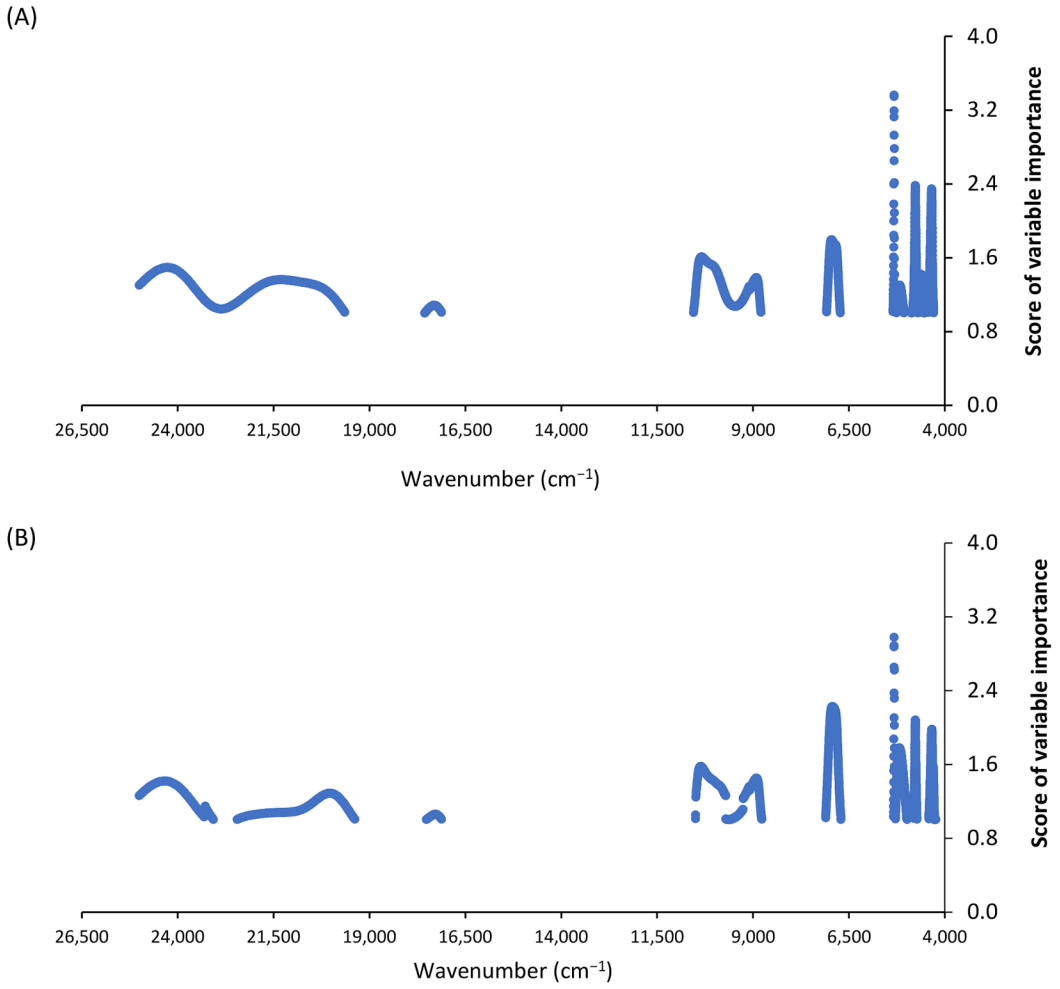


Figure 2. Blue dots represent the most important wavenumbers (variable importance >1; Caponigro et al., 2023) used for prediction of bovine colostrum (A) EAA and (B) mineral concentration via near-infrared spectroscopy.

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 affected 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-

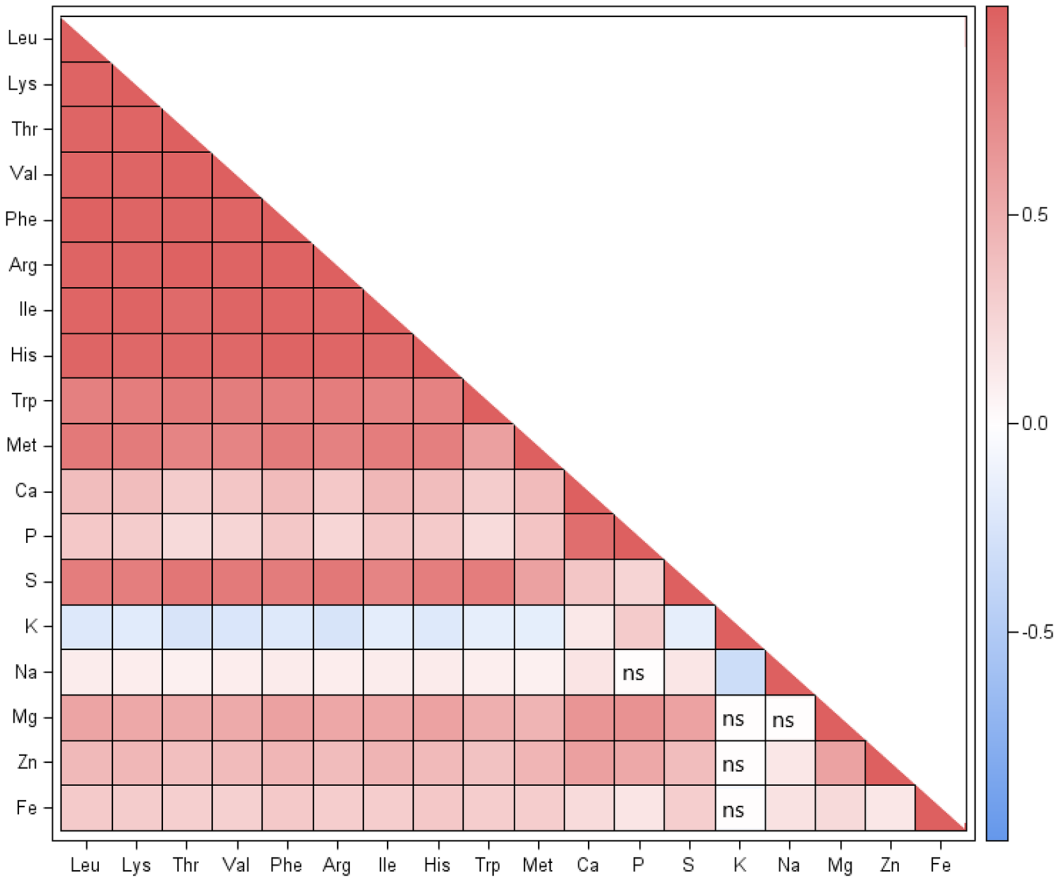


Figure 3. Heatmap with Pearson correlations ($P < 0.001$) between EAA and minerals of colostrum. Not significant coefficients are indicated with "ns."

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

	Parity			Calving season			
Trait	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

	Parity			Calving season			
Trait	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]	1945.57[b]	1901.71[b]	2033.55[a]	1,871.16[b]	2025.86[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 ($P < 0.05$).

[1]Log_{10}-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.

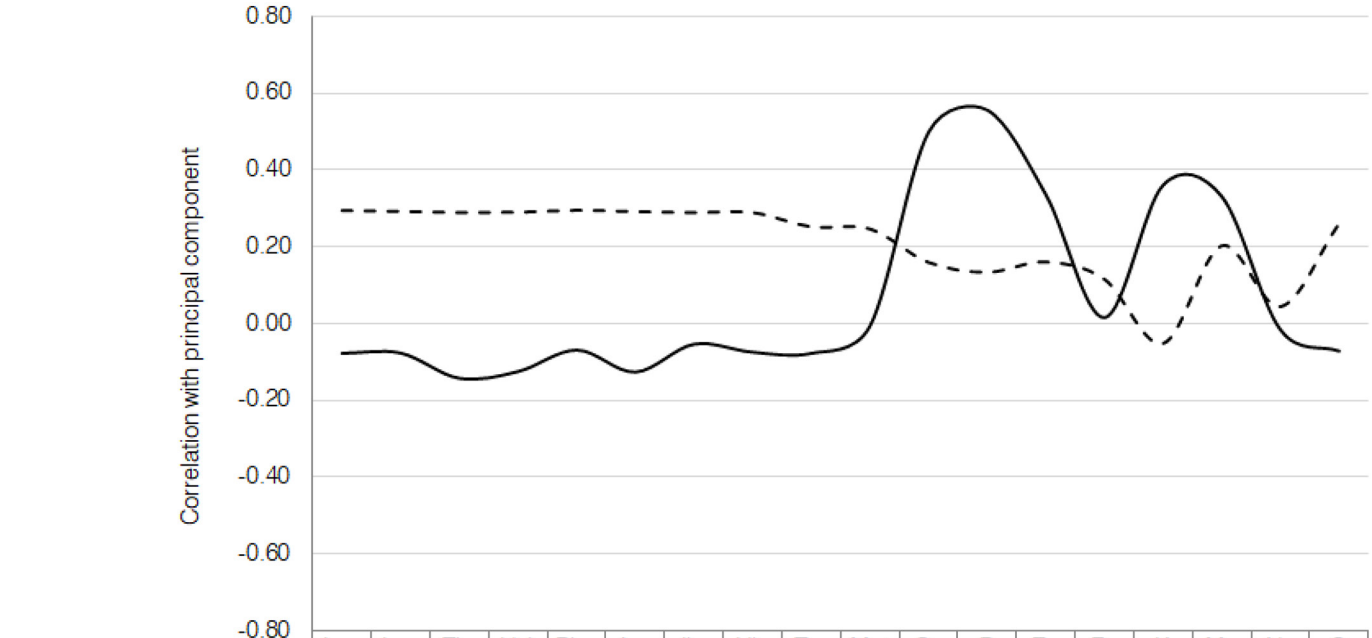
Detailed Composition and Ig Level

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 was 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.



	Leu	Lys	Thr	Val	Phe	Arg	Ile	His	Trp	Met	Ca	P	Zn	Fe	K	Mg	Na	S
Principal component 1	0.29	0.29	0.29	0.29	0.30	0.29	0.29	0.29	0.25	0.25	0.16	0.13	0.16	0.12	-0.05	0.20	0.04	0.26
Principal component 2	-0.08	-0.08	-0.14	-0.13	-0.07	-0.13	-0.05	-0.07	-0.08	-0.01	0.50	0.56	0.34	0.02	0.36	0.33	-0.02	-0.07

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

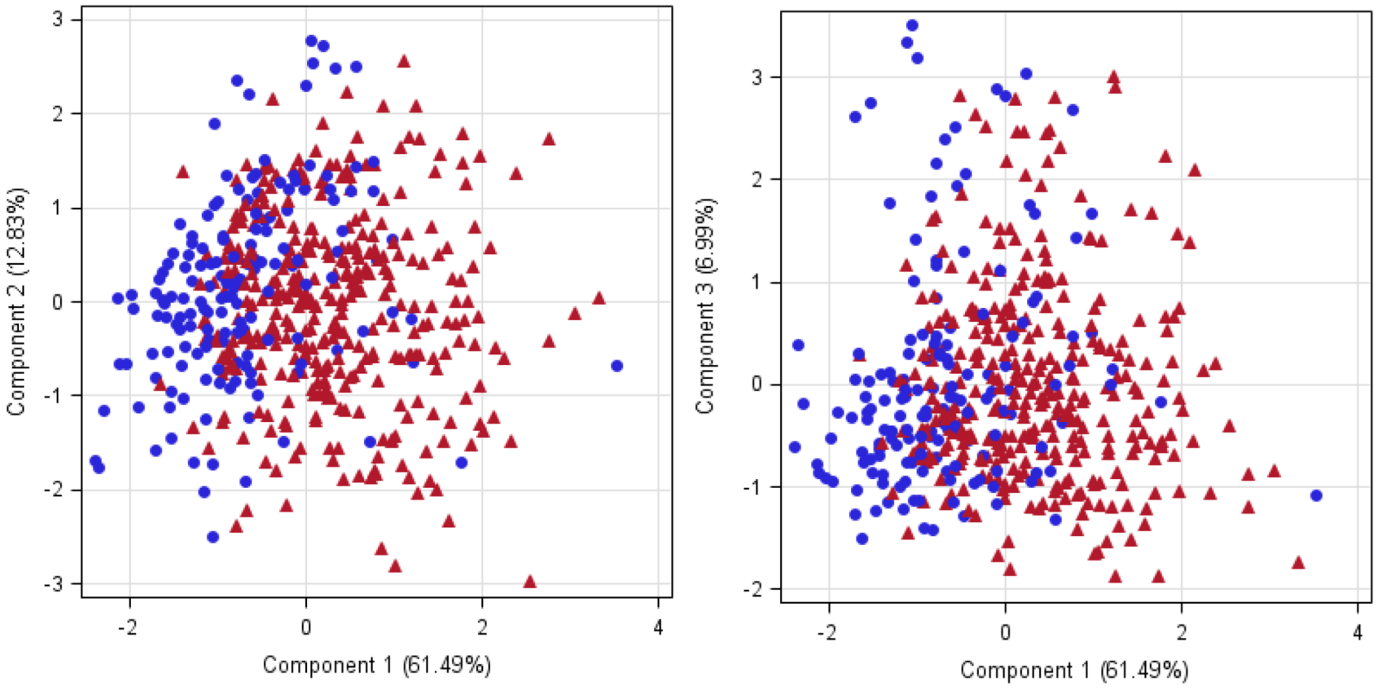


Figure 5. Graphical output of the scores of the principal components. Blue dots and red triangles represent low-quality (IgG <70 g/L; n = 159) and good-quality (IgG ≥70 g/L; n = 349) samples, respectively.

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