Infrared models for the prediction of cow colostrum immunoglobulins G concentration: phenotypic variability and relationship with colostrum yield

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Abstract

The concentration of immunoglobulins G (IgG) defines the narrow-sense quality of cows' colostrum. Administering colostrum with IgG < 50 g/L is not recommended for newborns due to the insufficient amount of antibodies and concrete risk of failure of passive transfer of immunity. In a former project, we investigated the predictive ability of FTIR for the prediction of IgG using 530 colostrum samples harvested within 6 h from parturition in 9 Holstein farms. To develop infrared prediction models radial immunodiffusion kits were used for IgG determination (gold method) and spectra were collected through FOSS machineries used for official milk analyses (FOSS A/S, Denmark). Regression-based approaches, from PLS up to machine learning algorithms, were used to train and test the models and prediction accuracies showing R^2 in external validation (R^2v) range from 0.70 to 0.85 and a root mean square error (RMSEv) around 13 g/L. With this background, the COLOXINF project was born in 2022 in collaboration with the Breeders Association of the Veneto Region (Italy). A total number of more than 4,000 samples were harvested in 95 farms following the previous protocol and the colostrum yield (CY, L) at first milking was recorded. IgG was predicted using the best performing model (R^2v = 0.84; RMSEv = 13.4 g/L) and, using exclusively purebred Holstein cows, we estimated IgG for each CY level (I: ≤3 L, II: 3-4 L, III: 4-6 L, and IV: ≥6.1 L). Prior to the statistical analyses, lactations were grouped (parity 1, 2, 3, 4, and ≥5), and samples with IgG deviating more than 3 SD from the mean and with CY outside the range 0.1-15.00 L were eliminated. The mixed model accounted for the random effect of the herd and fixed effect of parity, calving season (SEAS), CY level, and interactions of parity with both CY and SEAS. In a second analysis, we studied the variability of CY adjusting for parity, SEAS, and parity x SEAS interaction, with the herd as random. IgG and CY were normally distributed, averaged 102.16 g/L and 4.63 L, and were negatively correlated (-0.18). IgG was the lowest in primiparous (83.71±2.40 g/L) and the highest in parity 5 (117.15±2.75 g/L) and varied according to CY: 110.44±2.30, 104.95±2.48, 98.50±2.47, and 93.23±2.53 g/L for class I, II, III, and IV. Estimates suggested that there is dilution and that, regardless of the parity, low-producing cows (in class I) deliver colostrum with greater IgG, e.g., 17.21 g/L more compared to IV. The significant interaction between parity and CY demonstrated that the greatest IgG was provided by the less-yielding cows in parity 4 (123.30±3.32 g/L) and 5 (124.06±3.58 g/L): considering that at least 200 g of IgG must be provided to calves at the first meal, it derives that just 1.6 L of colostrum collected from these cows is sufficient to cover the requirements. As regards CY, the lowest and greatest yield were found in parity 1 (4.19±0.24 L) and 2 (5.42±0.26 L), with the latter being not significantly different from parity 3, 4, and 5.

Keywords: passive transfer of immunity, animal health, young stock survival, infrared spectroscopy, novel phenotype, immunoglobulin G.

Introduction

The quality of bovine colostrum relies on concentration of immunoglobulins G (IgG, g/L) which can be assessed through various lab methods that differ in terms of accuracy, costs, and facility of use and implementation. Colostrum administration procedures, the quantity delivered, and its IgG level determine the risk of passive transfer of immunity in calves. Colostrum with concentration of IgG below 50 g/L is of insufficient quality for neonates, particularly if the intake is less than 4 L. Moreover, ideally the first meal should occur as soon as possible after birth and no later than 6 h of life. Colostrum IgG has been studied widely in recent years, particularly in cattle. This trait is usually determined in the first colostrum (i.e. collected between 0 and 6 h after calving) using the gold standard radial immunodiffusion assay (RID). In the field, indirect measure of colostrum quality is provided by instruments like colostrometers and refractometers (Costa *et al.,* 2023). Other indirect methods may exploit the Fourier transform infrared spectroscopy (FTIR) and could be promising and accurate for IgG determination in colostrum (Franzoi *et al.,* 2022). In fact, FTIR represents the most popular machinery of milk official analyses and is successfully used worldwide. The present study aimed to evaluate if the narrow-sense colostrum quality – IgG concentration – can be predicted from the colostrum spectra collected by the Milkoscan FT7 (FOSS A/S, Denmark). For this purpose, various regression-based approaches, from partial least square regressions up to machine learning algorithms, were used to train and test the models. Subsequently, the best model was selected and used in a prediction set that consisted of 3,379 individual colostrum spectra. Samples were collected from 95 farms that joined the COLOXINF project funded by the Breeders Association of the Veneto Region (Italy) in January 2022. For each sample in the prediction set, the quantity of colostrum yielded at first milking (CY, L) was recorded.

Material and methods

FTIR performance

Data

The experimental design and sampling protocol have been widely described in Costa *et al.* (2021). The farmers involved in the first trial were 9 and provided the initial samples used for developing the prediction model. Using bovine-specific kits, colostrum IgG was determined as described by Costa *et al.* (2021) and the spectra were collected at the laboratory of ARAV (Vicenza, Italy) equipped with the Milkoscan FT7 (FOSS A/S, Denmark) on the same samples. The spectra acquisition took place on diluted aliquots (1 colostrum:1 pure deionized water).

Calibration and validation

Both the PLS and the Random Forest Ensamble method were used in regression for evaluating colostrum IgG predictability using the FTIR spectrum, net of water absorption regions, as predictor. The dataset was randomly split into a testing set (70%) and a validation set (30%) and the model performance in external validation included the coefficient of determination (R^2v) and the root mean square error (RMSEv). Preliminarily, spectral wavelengths in transmittance were converted to absorbance by taking the log10 of the reciprocal. A self-built script was created using the packages *tidyverse*, *caret* and *pls* available in the R software to carry out calibrations and model validations (Liaw andWiener, 2002; R Core Team, 2022).

Prediction set

Phenotypes

In January 2022 the COLOXINF project of the Breeders Association of the Veneto Region (ARAV, Vicenza, Italy) started in collaboration with the University of Padova. The aim of the project was to collect more than 4,000 colostrum samples to retrieve spectra and predict IgG using the most performing model developed earlier (i.e., with the best performance in external validation). Using the protocol adopted by farmers in the previous experiment, individual colostrum samples were collected in 95 farms for approximately 12 months. Only samples obtained within 6 h from calving were considered and, when possible, the CY of the donor cow at first milking was recorded.

Statistical analysis

Only purebred Holstein cows were retained (n = 2,728) and samples whose infrared-predicted IgG deviated more than 3 SD from the mean and with CY outside the range 0.1-15.00 L were removed. Prior to the statistical analyses, records were grouped for both parity (1, 2, 3, 4, and ≥5) and CY, with 4 levels identified: ≤3 L (I), 3-4 L (II), 4-6 L (III), ≥6.1 L (IV). An analysis of variance was used to evaluate the dilution effect of IgG by adjusting for the CY level. The mixed model accounted for the random effect of the herd and fixed effect of parity, calving season, CY level, and first-order interactions of parity with both CY and season. In a second analysis, the variability of CY was studied to estimate least squares means (LSM) for the fixed effects, namely parity, calving season, and parity x season interaction. Herd was included in the model as random.

Results

FT-MIR performance

Both the PLS and the Random Forest showed FTIR spectrum to be promising when attempting to predict the punctual concentration of IgG in cow's colostrum. While the first performed very good (R^2v = 0.85, RMSEv = 13 g/L), Random Forest, instead, resulted in a lower R^2v (0.74) although the same coefficient in training was high (0.97). When using Random Forest, predicted IgG values were more dispersed in testing than training (Figure 1) and the out-of-bag error, indicator of prediction error under bootstrapping, was equal to 0.08.

Given the apparent risk of overfitting with Random Forest, PLS was chosen for the IgG determination in the prediction set. According to the weight assigned to the wavelengths, the most important regions of the spectrum have been identified as suggested by Caponigro *et al.* (2023) using the variable importance in projection (VIP), where VIP scores > 1 belong to the most important predictors (Figure 2).

Variability of colostrum traits

Both IgG and CY were normally distributed, averaged 102.16 g/L and 4.63 L, and were negatively correlated (-0.18). IgG was significantly affected by all the fixed effects, except for the interaction between parity and CY level (P=0.061; Figure 3). IgG was the lowest in primiparous and the highest in oldest cows (Table 1). In the case of CY level, LSM of IgG were all significantly different, being equal to 110.44±2.30, 104.95±2.48, 98.50±2.47, and 93.23±2.53 g/L for class I, II, III, and IV. The analysis of variance revealed that autumn calving cows are those yielding the top-quality colostrum (Table 1).

Estimates given in Figure 3 suggest that there is a dilution effect. In particular, regardless of the parity, low-producing cows (CY class I) seem to deliver colostrum with greater IgG, i.e., with 17.21 g/L more compared to those in class IV. The LSM of the interaction between parity and CY (Figure 3) highlighted that the greatest amount of IgG was provided by the less-yielding cows in parity 4 (123.30±3.32 g/L) and 5 (124.06±3.58

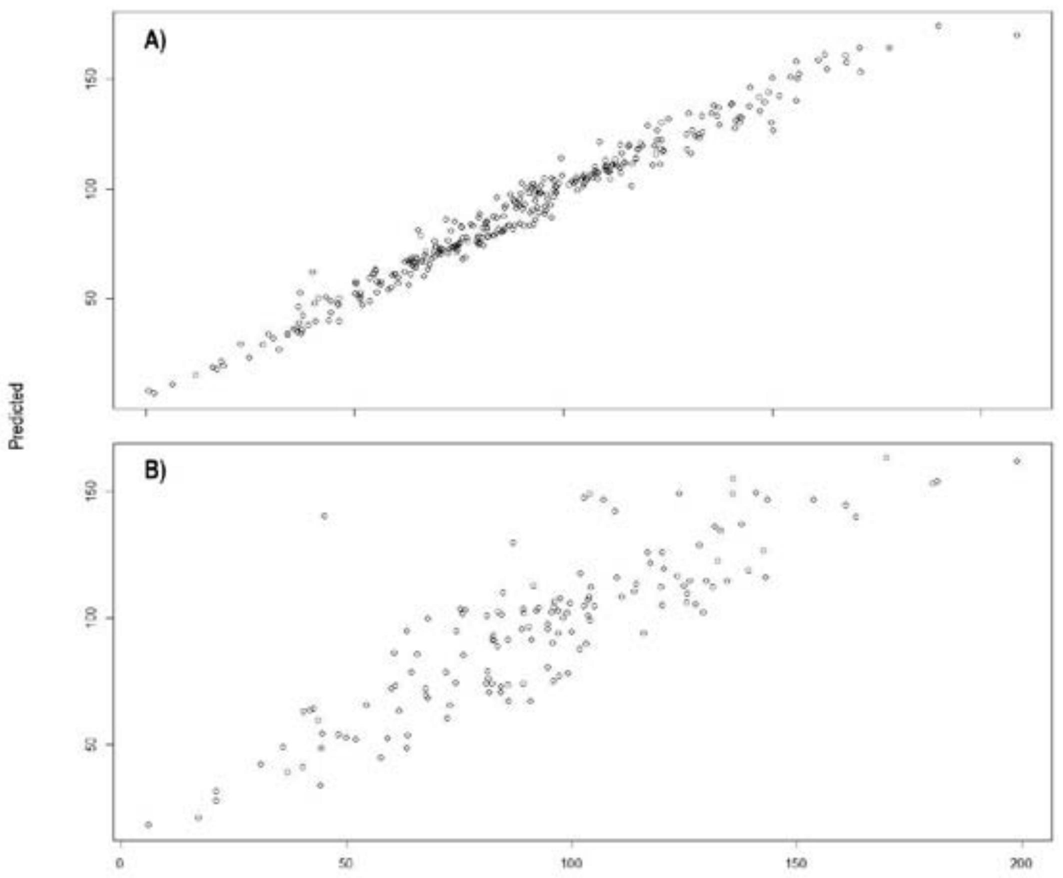

Figure 1. Predicted vs measured IgG concentration in A) training and B) testing set.

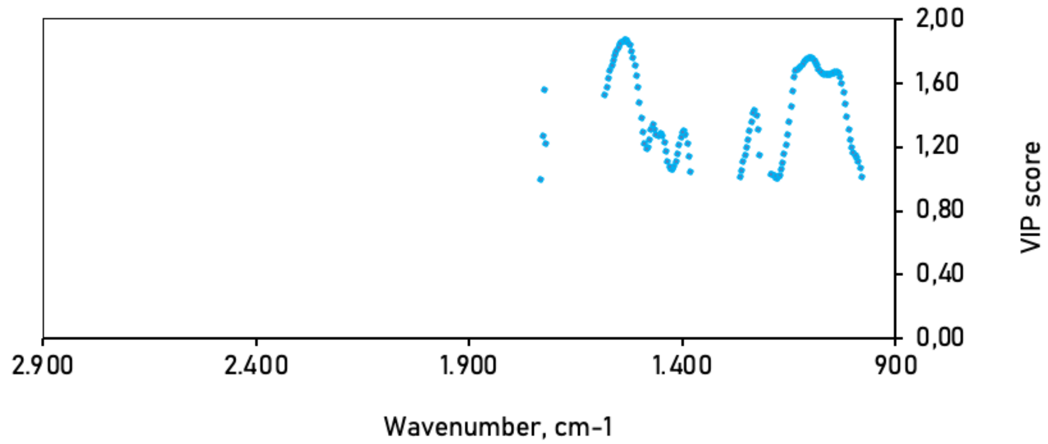

Figure 2. The most important (VIP score > 1) wavenumbers used for prediction of IgG via PLS.

Table 1. LSM[1] of parity and calving season estimated for IgG concentration (g/L) and CY (L).

	Parity					Calving season			
Trait	1	2	3	4	≥ 5	Winter	Spring	Summer	Autumn
IgG	83.71[d]	93.14[c]	105.06[b]	112.74[a]	117.15[a]	100.86[b]	98.45[ab]	102.84[b]	109.00[a]
CY	4.19[b]	5.42[a]	4.96[a]	4.93[ab]	4.89[ab]	4.48[b]	5.34[ab]	5.12[a]	4.50[b]

[1] Different letters within trait and within effect indicate significantly different estimates (P<0.05).

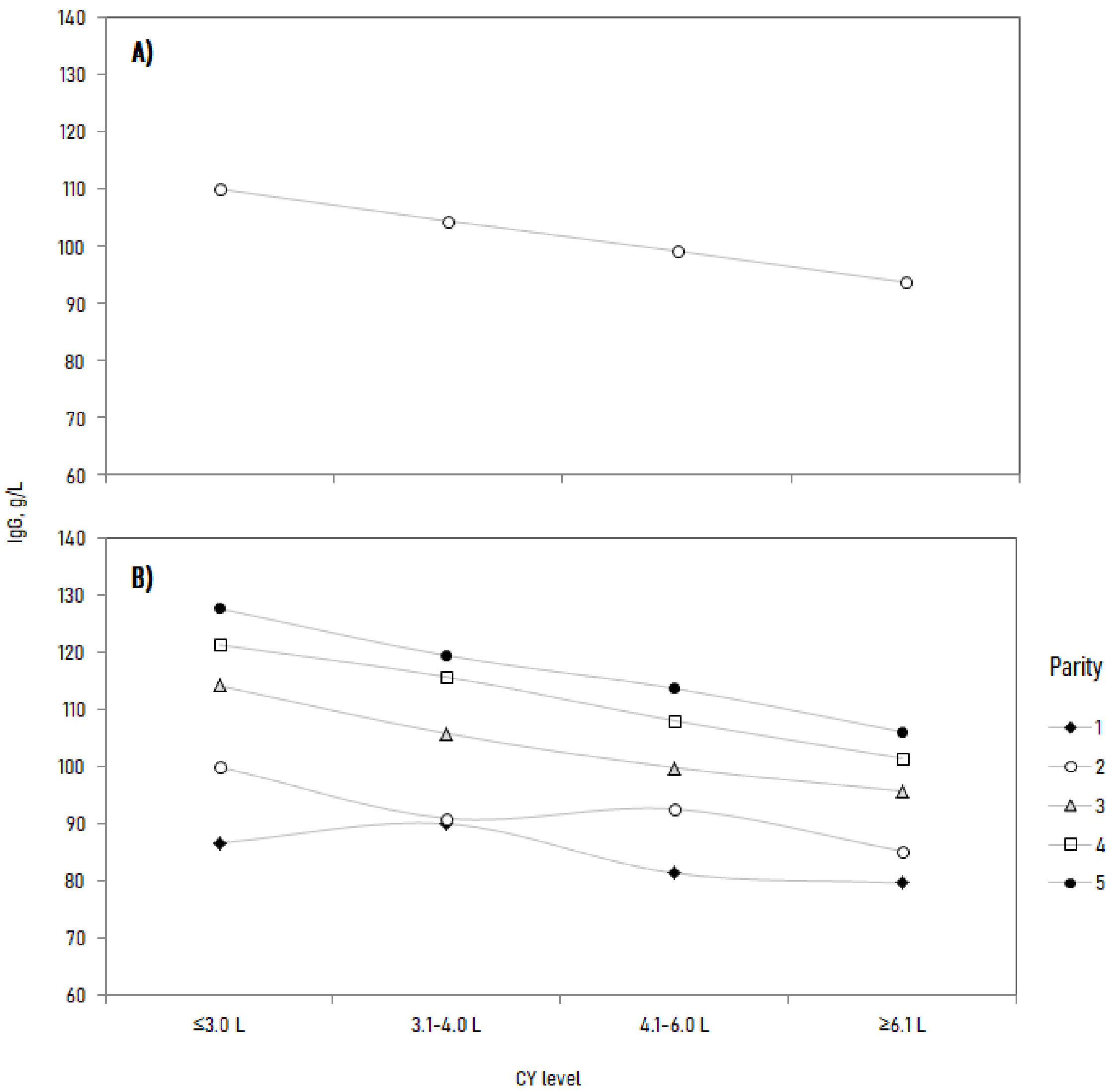

Figure 3. LSM of A) individual effect of CY level (P<0.001) and B) its interaction with parity (P=0.061) estimated for IgG concentration.

g/L). Considering that at least 200 g of IgG must be provided to calves at the first meal, it derives that just 1.6 L of colostrum collected from these cows can be considered as sufficient to cover the requirements. This volume is quite lower compared to the conventional amounts recommended (Godden *et al.,* 2019). In fact, considering that the minimum acceptable IgG concentration corresponds to 50 g/L, in general guidelines talk about at least 4 L of colostrum administered at first meal within 6 h from birth to satisfy the calf's IgG requirement (Godden *et al.,* 2019; Costa *et al.,* 2021).

Parity and calving season but not their interaction significantly affected CY (P=0.468). Estimated LSM are presented in Table 1 and indicate that the lowest and greatest amount of colostrum is found in first- (4.19±0.24 L) and second-lactation cows (5.42±0.26 L) and LSM of parity 3, 4, and 5 fall between them (Table 1). Cows calving in summer are those delivering more colostrum compared to other seasons, with on average 5.12 L delivered at first milking. These findings are in line with Gavin *et al.* (2018) who observed that cases where the parturient cow produces low CY are more frequent in winter. As reviewed by Costa *et al.* (2023), the photoperiod plays an important role in dairy cattle metabolism, regulations, and physiology, including lactogenesis. In fact, CY is reported to progressively increase with the number of light hours, i.e., in the period from spring to summer in the northern hemisphere (Zarei *et al.,* 2017; Gavin *et al.,* 2018; Costa *et al.,* 2023).

The present study opens the room for the use of FTIR for colostrum IgG prediction and paves the way for more studies willing to explore the relationship between IgG concentration and CY in dairy cows. In fact, this can be considered as the first study attempting to investigate the variability of CY at first milking (<6 h from calving) on a large number of animals and herds adopting a standard protocol.

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