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

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Introduction
Colostrum

• First secretion of mammary gland
• Provides bioactive factors, nutrients, and antibodies to the neonate
• Progressive changes in composition in the first h after calving
• Very different density/composition compared to mature milk

Generally, an optimal passive transfer of immunity relies on 3Q

Quickness ➔ within 6 h of life
Quantity ➔ at least 4 L
Quality ➔ >50 g/L immunoglobulins G (IgG)
Calf

[ IgG ] calf serum

Calf's gut permeability

[ IgG ] colostrum

Percentage to maximum

0 12 24 36 48 60
h post-partum

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Calf

Collecting the very first colostrum is recommended to feed calves and/or for banking (farm colostrum bank).

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IgG assessment

Gold standard:
- Radial immunodiffusion (RID) assay
- ELISA test

Time consuming / very expensive / high skilled technicians

Infrared technology
- Milk DHI testing system
- Low cost
- Time-saving (>300 samples/h)
- A posteriori prediction from stored spectra
Aims

1. To demonstrate predictive ability of infrared spectroscopy for the prediction of colostrum IgG

2. To evaluate infrared colostrum prediction models on-field and to investigate the relationships between volume of colostrum yield and IgG in Holstein Friesian
Materials & Methods
The rationale

Develop FTIR prediction model for IgG

n. final samples > 500
The rationale

Develop FTIR prediction model for IgG

FTIR calibration for IgG

n. final samples > 500
The rationale

Develop FTIR prediction model for IgG

Routine samples collection and prediction of IgG

FTIR calibration for IgG

n. final samples > 500

n > 4,000

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Methods - FTIR model development

- 678 cows - 9 HF farms - 15-mo period
- Colostrum samples (stored in plastic sterile tubes - 120 mL) collected by farmers within 6 h from parturition and immediately frozen (-20 °C)
- Periodically, samples picked up and transferred to the lab of the University of Padua for quality assessment
- Cow ID and calving date indicated on the tubes

(Costa et al., 2021; Costa et al., 2022; Goi et al., 2023)
Methods - FTIR model development

Reference

- IgG (+other fractions like IgA and IgM) quantified by radial immunodiffusion kits specific for bovine (Triple J Farms - Bellingham, WA, US).
- RID repeatability tested (Costa et al., 2021)
Methods - FTIR model development

Reference

- Protein and fat content determined through Kjeldahl (AOAC, 2000) and VDLUFA (VDLUFA, 2013)
- Mineral composition Ca, P, S, K, Na, Mg, Zn, and Fe quantified by ICP-OES
- Amino acids composition (Leu, Lys, Thr, Val, Phe, Arg, Ile, His, Met) by reversed-phase HPLC + UV detection

(Goi et al., 2023)
Methods - FTIR model development

Spectra

- FTIR - 25 mL of colostrum were diluted (1:1) in pure water and analyzed with MilkoScan 7 RM (FOSS Electric A/S, Hillerød, Denmark) → wavelength range between 5,011.54 and 925.92 cm\(^{-1}\)
- NIRS - 10 mL of colostrum scanned with DS2500 (FOSS Electric A/S, Hillerød, Denmark) → wavelength range between 25,000 to 4,000 cm\(^{-1}\)
Methods - FTIR model development

Chemometric analysis

- Quality control: elimination of outliers in both references and spectra + deletion of water absorption regions
- Standard normal variate (SNV) scatter correction on spectra
- PLS regression analysis - self-built macro (SAS software v. 9.4, SAS Institute Inc., Cary, NC, USA)
## Results - FTIR model development

### Descriptive statistics of colostrum IgG measured by RID

<table>
<thead>
<tr>
<th>Trait</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG, g/L</td>
<td>531</td>
<td>93.54</td>
<td>33.87</td>
<td>9.22-198.90</td>
<td>36.21</td>
</tr>
</tbody>
</table>

### Abstract

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### Fitting statistics* of PLS in 5-fold cross-validation and external validation for IgG

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>Outliers, %</th>
<th>LV</th>
<th>RMSE&lt;sub&gt;CV&lt;/sub&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;&lt;sub&gt;CV&lt;/sub&gt;</th>
<th>n&lt;sub&gt;ext&lt;/sub&gt;</th>
<th>RMSE&lt;sub&gt;V&lt;/sub&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;&lt;sub&gt;V&lt;/sub&gt;</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG, g/L</td>
<td>383</td>
<td>4.0</td>
<td>10</td>
<td>9.53</td>
<td>0.92</td>
<td>132</td>
<td>13.39</td>
<td>0.84</td>
<td>2.49</td>
</tr>
</tbody>
</table>

* n = final samples used in calibration; LV = n. latent variables; RMSE<sub>CV</sub> = root mean square error in cross-validation; R<sup>2</sup><sub>CV</sub> = coeff. determination in cross-validation; n<sub>ext</sub> = final samples used in external validation; R<sup>2</sup><sub>V</sub> = coeff. determination in external validation; RPD = residual predictive deviation calculated as st. dev. of reference data (validation) to st. error of predictions.
Results - FTIR model development

Plot of predicted vs reference IgG (external validation)

The most important wavenumbers (predictors)
## Results - FTIR model development

### Descriptive statistics of reference and predicted IgG in calibration and validation sets

<table>
<thead>
<tr>
<th>Trait</th>
<th>Determination</th>
<th>Dataset</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG, g/L</td>
<td>RID</td>
<td>Calibration</td>
<td>399</td>
<td>93.55</td>
<td>34.11</td>
<td>9.22-198.90</td>
<td>36.46</td>
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<tr>
<td></td>
<td></td>
<td>Validation</td>
<td>132</td>
<td>93.50</td>
<td>33.28</td>
<td>21.48-181.10</td>
<td>35.59</td>
</tr>
<tr>
<td></td>
<td>FTIR</td>
<td>Calibration</td>
<td>383</td>
<td>92.40</td>
<td>31.55</td>
<td>7.31-177.64</td>
<td>34.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Validation</td>
<td>132</td>
<td>93.51</td>
<td>30.98</td>
<td>8.06-169.35</td>
<td>33.13</td>
</tr>
</tbody>
</table>
Methods - FTIR model application

• > 4,000 (HF, SI, Rendena) cows in 95 dairy farms
• ColoXInf project of Breeders Association of Veneto Region - A.R.A. Veneto
• Colostrum samples collected using previous protocol at first milking (< 6 h)
• Colostrum yield (CY, L) at first milking recorded
• IgG FTIR-predicted

• Editing: only HF (n = 2,728) + outliers deletion
• 4 CY levels: • group I: ≤3 L • group III: 4-6 L
  • group II: 3-4 L • group IV: ≥6.1 L
• Mixed model: fixed effect of parity, calving season, CY level, parity x CY level, season x CY level. Herd random.
Methods - FTIR model application

Descriptive statistics of predicted IgG and CY

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>SD</th>
<th>Range after editing</th>
<th>CV, %</th>
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</thead>
<tbody>
<tr>
<td>IgG, g/L</td>
<td>102.16</td>
<td>33.62</td>
<td>2.07 - 209.96</td>
<td>32.90</td>
</tr>
<tr>
<td>CY, L</td>
<td>4.63</td>
<td>2.28</td>
<td>0.10 - 15.00</td>
<td>49.20</td>
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Frequency distribution IgG

Frequency distribution CY

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# Methods - FTIR model application

## LSM of IgG

<table>
<thead>
<tr>
<th>Class Colostrum Yield</th>
<th>IgG, g/L</th>
<th>St. Err.</th>
<th>L. colostrum needed to deliver 200 g. IgG</th>
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<tr>
<td>I LOW - YIELDING</td>
<td>110,02 A</td>
<td>2,31</td>
<td>1,82</td>
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<tr>
<td>II</td>
<td>104,45 B</td>
<td>2,51</td>
<td>1,98</td>
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<tr>
<td>III</td>
<td>99,18 C</td>
<td>2,51</td>
<td>2,02</td>
</tr>
<tr>
<td>IV HIGH - YIELDING</td>
<td>93,71 D</td>
<td>2,54</td>
<td>2,14</td>
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## Methods - FTIR model application

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Methods – FTIR model application

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Take home messages
**Conclusion**

- FTIR accurately predicts bovine colostrum IgG
- IgG punctual data points are phenotypes potentially useful to:
  - Farmers
  - Breeders
  - Food/pharma/dairy industry
On-going practical applications

- Italian laboratory (ARAV, Vicenza) provides colostrum IgG and gross composition by infrared (6 euro/sample)

- Italian Holstein Breeders Association (ANAFIBJ) is working on colostrum genetic and genomic index based on predicted IgG
Further activities

• Explore other colostrum traits (e.g., minerals, amino acids)

• Other technologies
  • pocket infrared tools for at farm application
  • X-ray to improve mineral composition prediction

• Investigate the current calf/colostrum management practices (> 500 farmers interviewed so far)

• Understand the relationship between colostrum quality and cow welfare (e.g., Classyfarm scoring system)
Thank you!

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