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

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Introduction

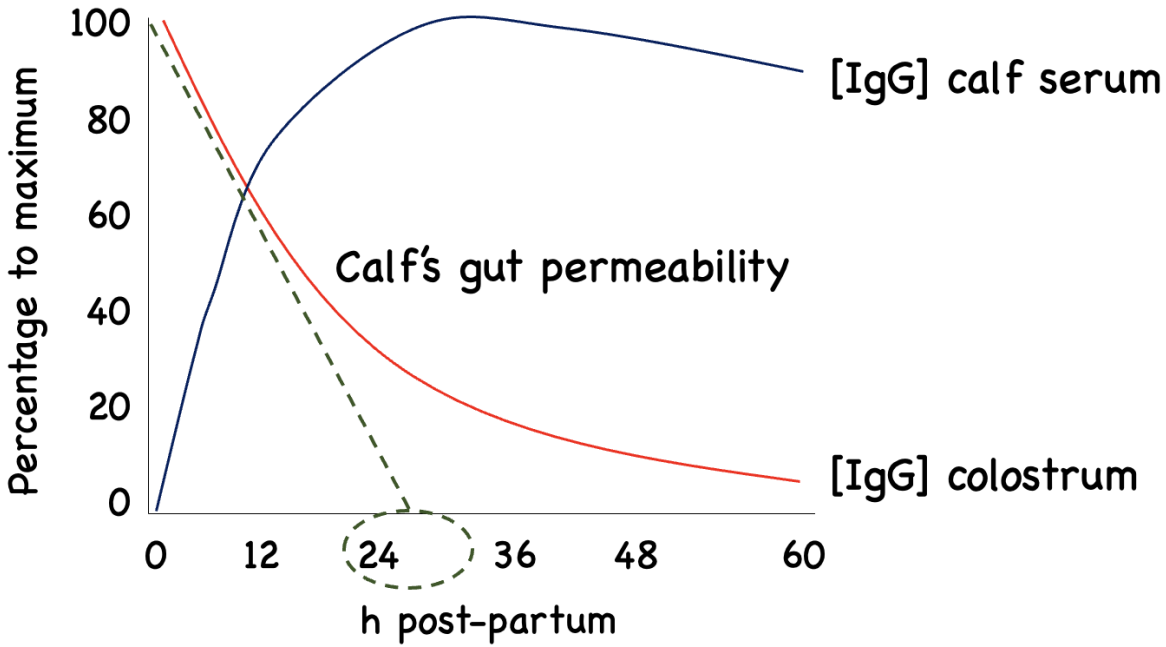
Colostrum

- 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

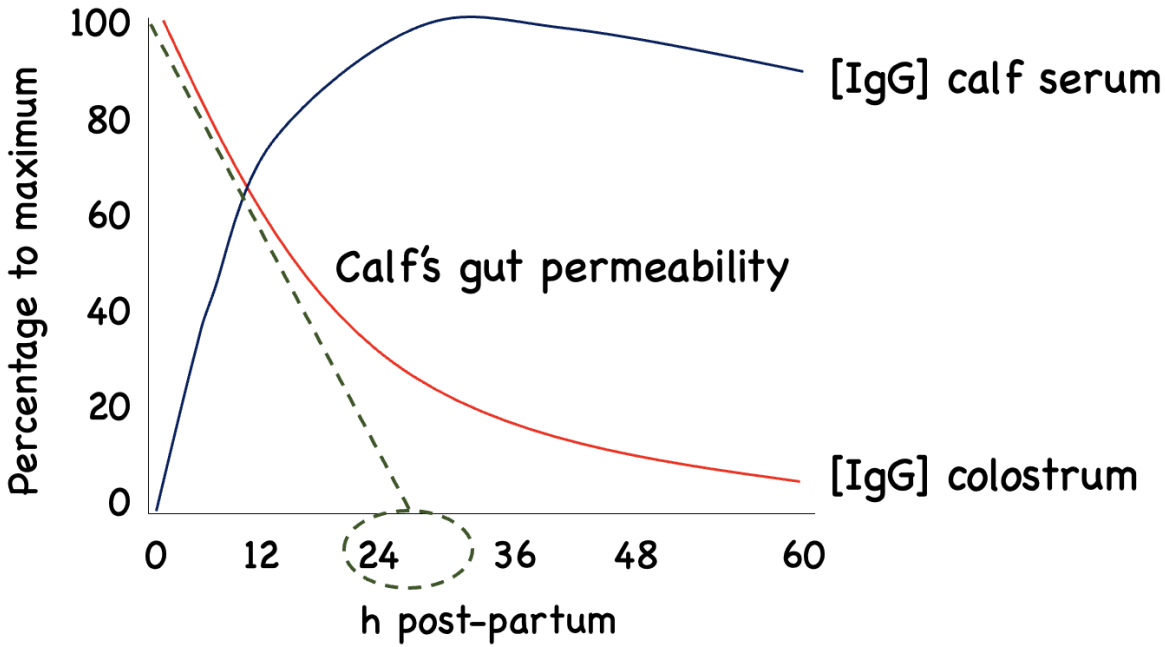
Generally, an optimal passive transfer of immunity relies on 3Q

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

Calf


100
80
60
40
20
0
Percentage to maximum
0
12
24
36
48
60
h post-partum
[IgG] calf serum
Calf's gut permeability
[IgG] colostrum

Calf



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

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

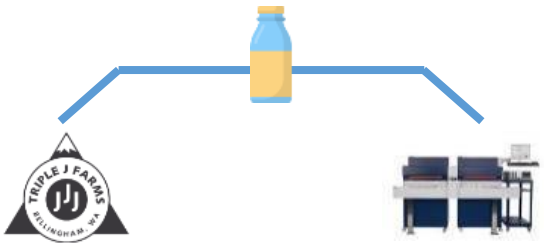
Aims

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

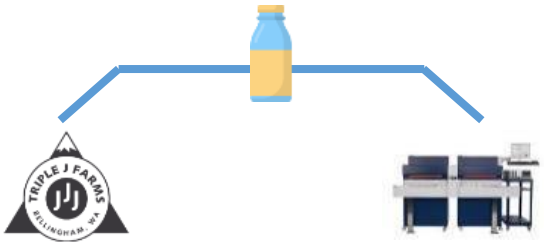
Develop FTIR prediction model for IgG



n. final samples > 500

The rationale

Develop FTIR prediction model for IgG

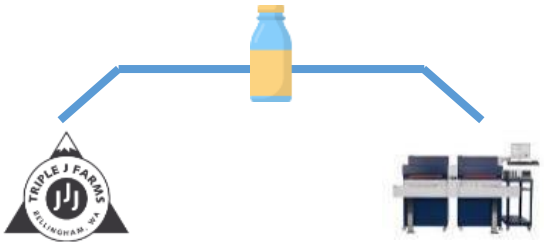


n. final samples > 500

FTIR calibration for IgG

The rationale

Develop FTIR prediction model for IgG



n. final samples > 500

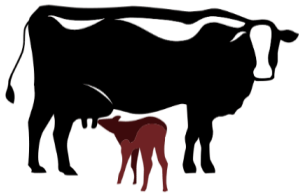
FTIR calibration for IgG

Routine samples collection and prediction of IgG

n > 4,000

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

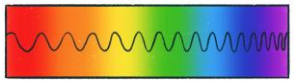
- Protein and fat content determined through Kjeldahl (AOAC, 2000) and VDLUFA (VDLUFA, 2013)
- Mineral composition Ca, P, S, K, Na, Mg, Zn, and Fe quantified by ICP-OES
- Amino acids composition (Leu, Lys, Thr, Val, Phe, Arg, Ile, His, Met) by reversed-phase HPLC + UV detection

(Goi et al., 2023)

Methods - FTIR model development

Spectra

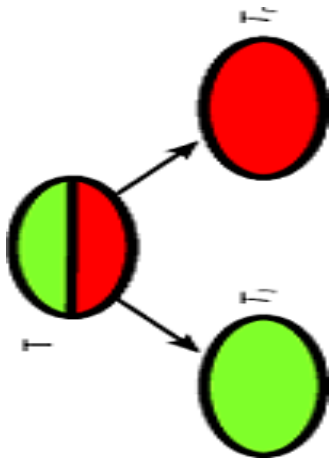
- FTIR - 25 mL of colostrum were diluted (1:1) in pure water and analyzed with MilkoScan 7 RM (FOSS Electric A/S, Hillerød, Denmark) →wavelength range between 5,011.54 and 925.92 cm^{-1}
- NIRS - 10 mL of colostrum scanned with DS2500 (FOSS Electric A/S, Hillerød, Denmark) →wavelength range between 25,000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



PLS - Calibration set (random 75%)

Model development + Internal validation

PLS - External validation set (random 25%)

Test the model using masked data

Results - FTIR model development

Descriptive statistics of colostrum IgG measured by RID

Trait	N	Mean	SD	Range	CV, %
IgG, g/L	531	93.54	33.87	9.22-198.90	36.21

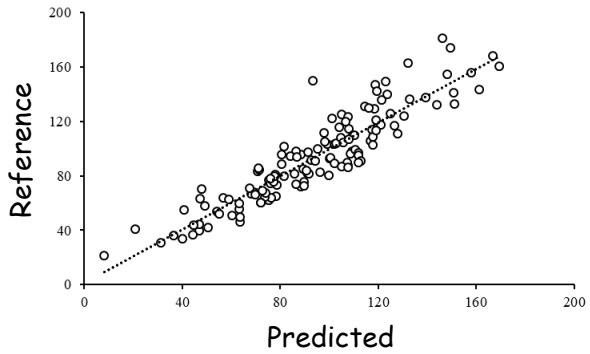
Trait	n	Outliers, %	LV	$RMSE_{CV}$	R^2_{CV}	n_{ext}	$RMSE_V$	R^2_V	RPD
IgG, g/L	383	4.0	10	9.53	0.92	132	13.39	0.84	2.49

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

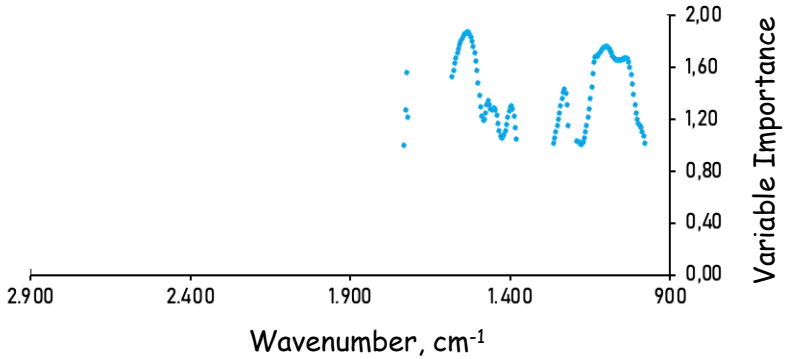
* n = final samples used in calibration; LV = n. latent variables; $RMSE_{CV}$ = root mean square error in cross-validation; R^2_{CV} = coeff. determination in cross-validation; n_{ext} = final samples used in external validation; R^2_V = coeff. determination in external validation; RPD = residual predictive 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

Trait	Determination	Dataset	n	Mean	SD	Range	CV, %
IgG, g/L	RID	Calibration	399	93.55	34.11	9.22-198.90	36.46
		Validation	132	93.50	33.28	21.48-181.10	35.59
	FTIR	Calibration	383	92.40	31.55	7.31-177.64	34.15
		Validation	132	93.51	30.98	8.06-169.35	33.13

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

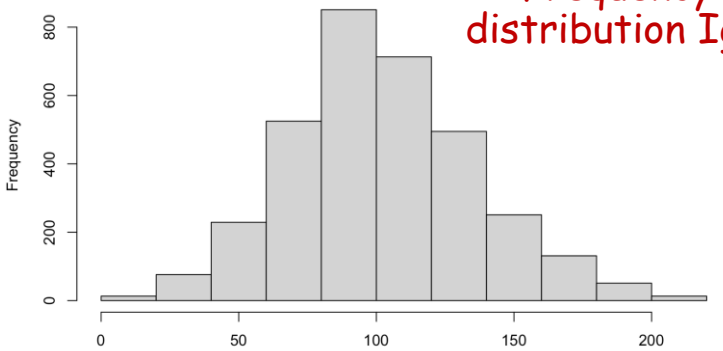
- Editing: only HF (n = 2,728) + outliers deletion
- 4 CY levels:
  - group I: ≤3 L
  - group II: 3-4 L
  - group III: 4-6 L
  - group IV: ≥6.1 L
- Mixed model: fixed effect of parity, calving season, CY level, parity x CY level, season x CY level. Herd random.

Methods – FTIR model application

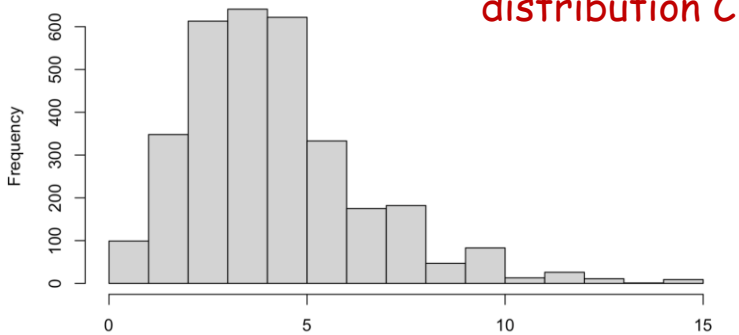
Descriptive statistics of predicted IgG and CY

Trait	Mean	SD	Range after editing	CV, %
IgG, g/L	102.16	33.62	2.07 – 209.96	32.90
CY, L	4.63	2.28	0.10 – 15.00	49.20

Frequency distribution IgG



Frequency distribution CY



Methods – FTIR model application

LSM of IgG

Class Colostrum Yield	IgG, g/L	St. Err.	L colostrum needed to deliver 200 g IgG
• I LOW - YIELDING	110,02 A	2,31	1,82
• II	104,45 B	2,51	1,95
• III	99,18 C	2,51	2,02
• IV HIGH - YIELDING	93,71 D	2,54	2,14

Methods – FTIR model application

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Methods – FTIR model application

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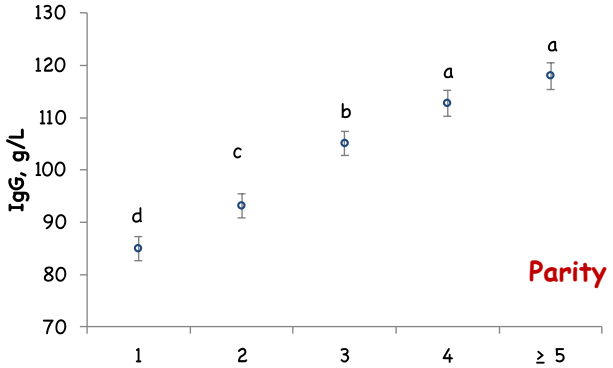

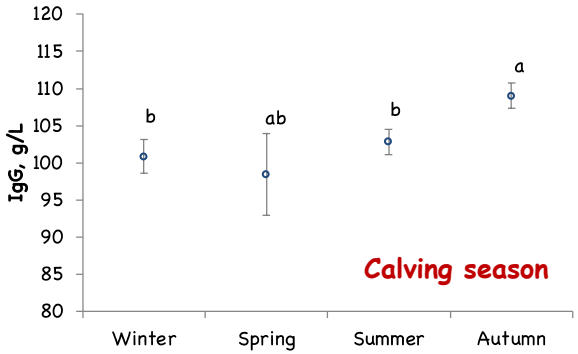

Take home messages

Conclusion

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

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Invited review: **Bovine colostrum, a promising ingredient for humans and animals—Properties, processing technologies, and uses**

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Costa et al. Genet Sel Evol (2021) 53:87
https://doi.org/10.1186/s12711-021-00681-8

GSE Genetics Selection Evolution

RESEARCH ARTICLE Open Access

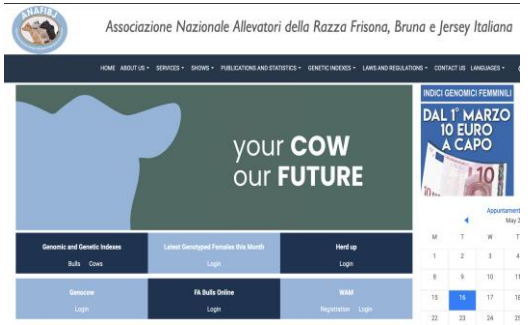
The concentrations of immunoglobulins in bovine colostrum determined by the gold standard method are genetically correlated with their near-infrared prediction

Angela Costa[1], Marco Franzoi[1*], Giulio Visentin[2], Arianna Goi[1], Massimo De Marchi[1] and Mauro Penasa[1]

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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