



ICAR - IDF meeting
Anand

ExtraMIR Workshop

31th March 2025



Agenda

08:30 – 08:45 : ICAR/IDF collaboration

08:45 – 09:15 : Introduction to the ExtraMIR project

09:15 – 09:35 : WP1- Reference method comparison

09:35 – 09:55 : Discussion on WP1

09:55 – 10:15 : WP2- Modelling

Break

11:00 – 11:20 : Discussion on WP2

11:20 – 11:40 : WP3 – Quality assurance

11:40 – 12:00 : Discussion on WP3

12:00 – 12:20 : WP4 communication

12:20 – 12:30 : General Discussion

ExtraMIR
(**Extra** value from- smart use of -**MIR** spectra)

Let's us present you the project started in 2022 ...



ICAR/IDF collaboration



10-6-2026

Silvia Orlandini (ICAR) and Daniel Nunez Diaz (IDF)

IDF GLOBAL REACH ALONG THE DAIRY CHAIN



- Established in 1903
- **41 member countries** representing **2/3 of world milk production** with a network of **1200 experts** (National Committees)
- Accredited by FAO, Codex, WOAHA, UNEP...
- Collaborates with NGO's (ISO, WFO, DSF, EDA, GDP, ICAR, FEPAL, OECD, etc.)



THE GLOBAL STANDARD FOR LIVESTOCK DATA

Network. Guidelines. Certification.

ICAR MEMBERS & ACTIVITIES AROUND THE WORLD

ICAR Members organisations • 135 members in 50 countries



SUBCOMMITTEES & WORKING GROUPS

- Animal Identification
- Measuring, recording and sampling devices
- **Milk analysis**
- Interbull
- Beef
- DNA
- Dairy cattle milk recording
- Conformation recording
- Feed intake and Greenhouse emissions
- Functional traits
- Artificial insemination
- Animal data exchange (ADE)
- Sheep, goats and camelids

ICAR/IDF Collaboration



- **Why IDF and ICAR work together?**

Complementary roles:

IDF: global reference body for dairy science, standards, and policy

ICAR: expertise in animal data, recording systems, and farm-level performance

Shared objective:

Ensure robust, comparable and scientifically sound data frameworks, with a focus on strengthening science-based approaches and harmonization across the dairy chain

Collaboration enables:

Alignment between farm-level data and global dairy standards

Improved credibility and consistency of sector approaches

Formalized by MoU in 2023, to ensure coordination and avoid duplication of work

10-6-2026

Reference System for Somatic cell Counting (RSSCC)



ICAR Milk Analyses SC

IDF SC Laboratory Statistics and Quality Assurance



- Main activities: Newsletter, monitoring the use of the gold standards (questionnaire), monitoring the store of the Reference Material together with EU JRC
- Main outputs: IDF/ICAR Bulletin (use of gold standard), revision of IDF/ISO standards on the way

Initiative on use of sensor data



ICAR Functional Traits - WG

IDF SC Animal Health and Welfare

- Active joint project
- Collaboration on topics addressing sensor data and its use for functional traits.
- Activities: exchange of information, joint publications and guidelines, presentation in Verona

IDF–ICAR Collaboration on ExtraMir

Joint approach



- ExtraMir is implemented as a joint initiative between IDF and ICAR, ensuring close collaboration throughout
- Work is conducted within the respective organisational structures and procedures of both IDF and ICAR
- Ensured by Silvia Orlandini (ICAR) and Aurélie Dubois (IDF)
- Members from both ICAR members (organisations, companies), and IDF members (National Committee: 1 member per country, represented by experts from the dairy sector)
- Aligned outputs:
 - In IDF: output are the voice of the global dairy sector (consensus among experts).
 - In ICAR: global standard for livestock data



How IDF and ICAR Work Together Within Their Respective Structures

Element	Role in ExtraMir	Organisation
WP1–WP4	Joint technical work packages forming the Action Team “ExtraMIR”	IDF & ICAR (joint)
Action Team “ExtraMIR” (Under the aegis of SCLSQA)	Collaborative platform for development of outputs	IDF & ICAR (joint)
MASC – Milk Analysis Subcommittee	Review and endorsement of outputs within ICAR governance	ICAR
SC on Laboratory Statistics and Automation	Review and follow-up of outputs within IDF governance	IDF
SC on Analytical Methods for Composition	Specialised review of WP1 outputs related to fat methods	IDF



ExtraMIR Governance & Collaboration Structure

Joint Collaboration Framework

- Structured partnership between International Dairy Federation (IDF) and International Committee for Animal Recording (ICAR)
- Combines technical expertise while preserving each organisation's governance and procedures

Central Technical Platform: Action Team "ExtraMIR"

- Joint IDF–ICAR expert group
- Organised into **4 Work Packages (WP1–WP4)**
- Collaborative development of methods, data sharing, and aligned technical outputs

Special Case: WP1 – Fatty Acids

- WP1 remains fully integrated within ExtraMIR
- Additional review within IDF through **SCAMC**
- Provides specialised oversight for fatty acid methodologies

Governance & Validation Pathways

ICAR route: Outputs reviewed through **MASC** (Milk Analyses Sub Committee)

IDF route: Outputs reviewed through **SCLSQA** (Standing Committee on Laboratory Standards and Quality Assurance)

IDF route: Outputs reviewed through SC **AMC** (Standing Committee on Analytical Main Component)

Ensures formal endorsement and integration within both organisations

Joint technical development + independent organisational governance

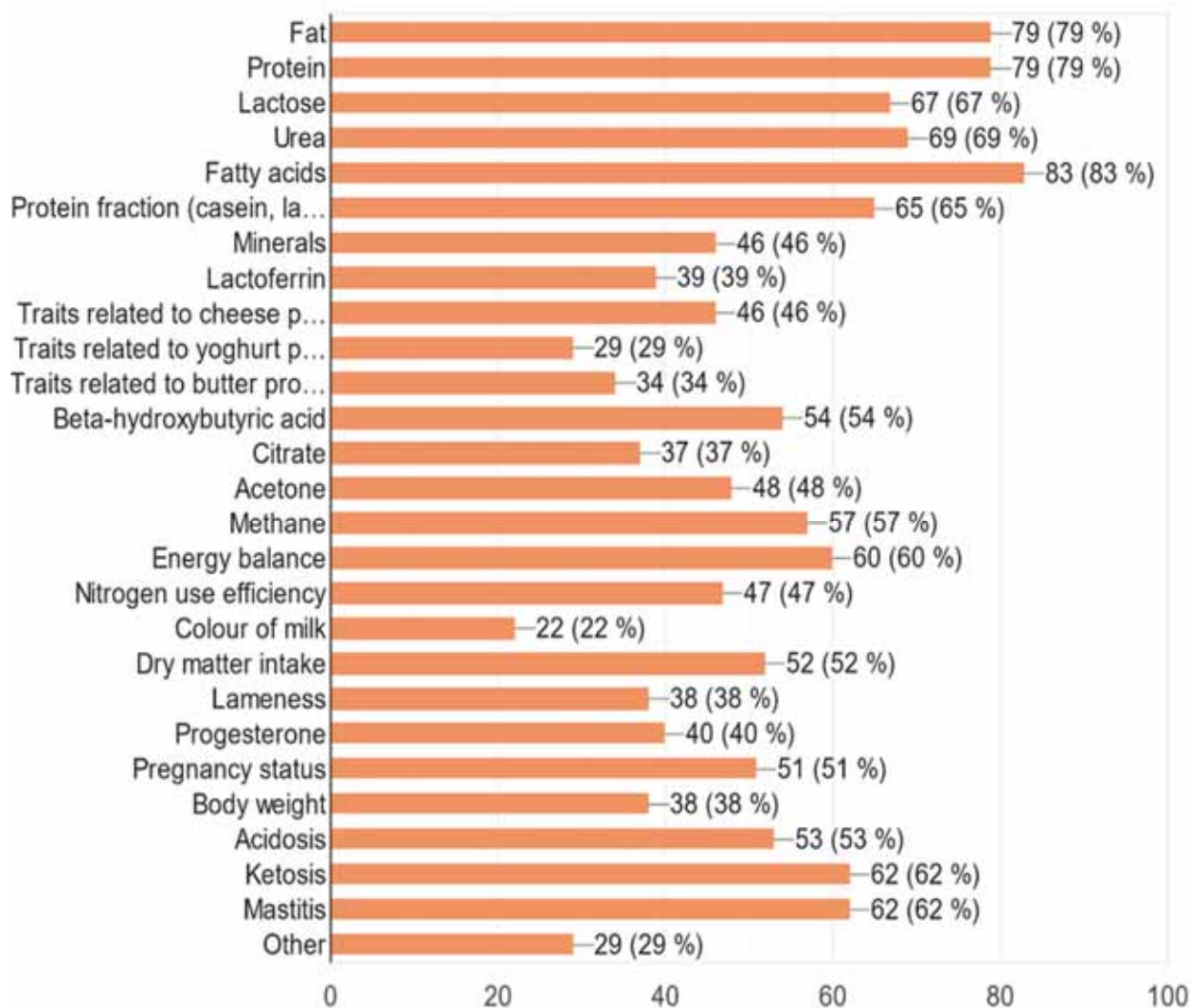
- Efficiency
- Institutional legitimacy
- Scientific robustness
- Long-term adoption of outcomes

Fatty Acids

Which interests ?

Relevant MIR-based predictions ?





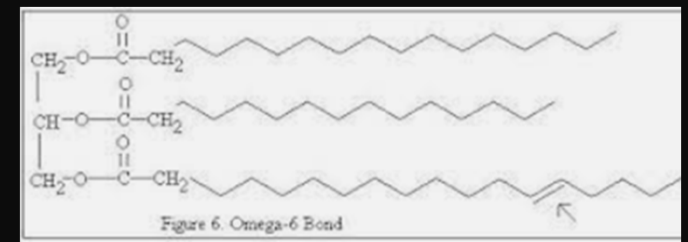
Why Fatty Acids ?



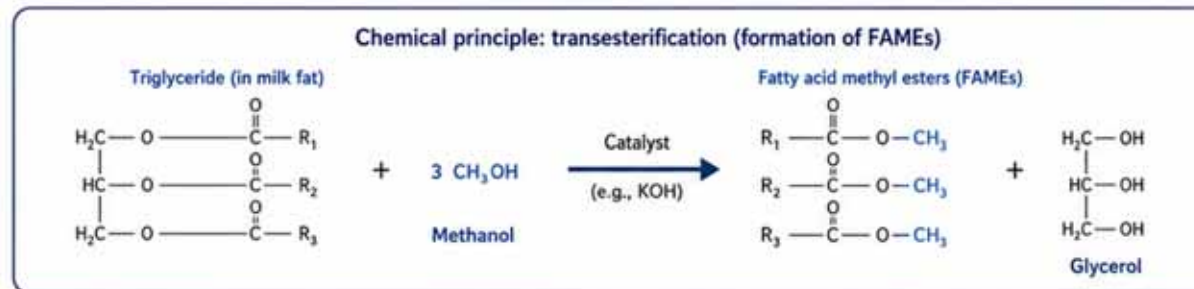
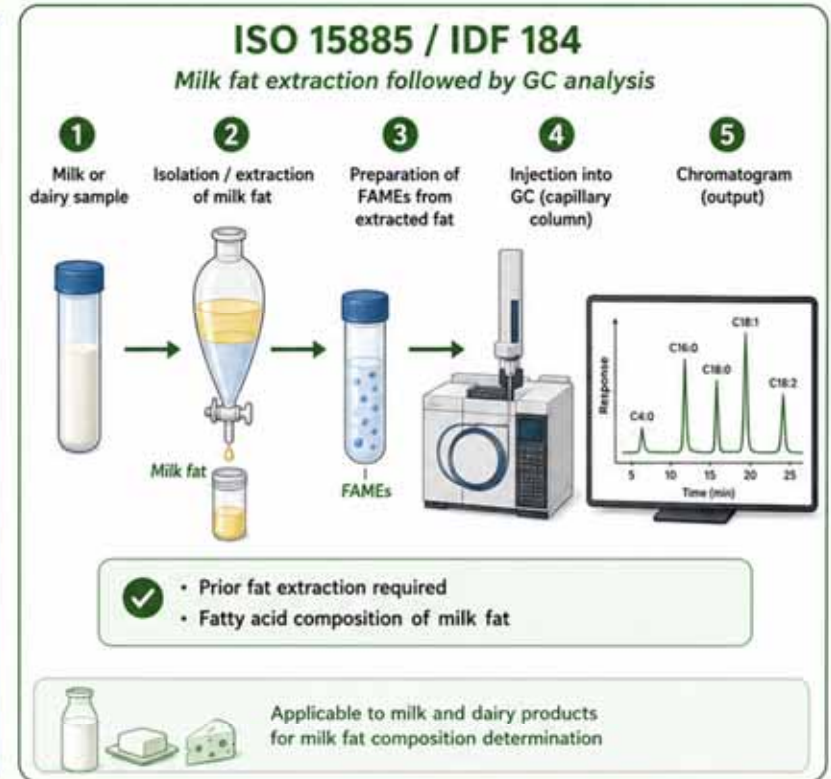
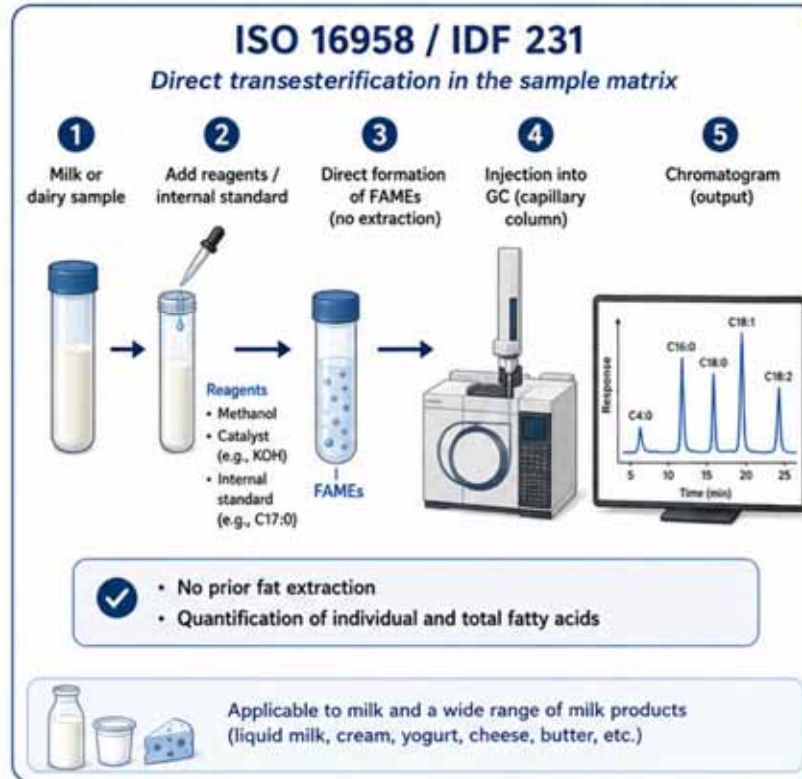
- 106 respondents
- 59.4% want to actively participate in the project

Milk - Fatty acids

- Milk is a natural emulsion of fat suspended in water (= fat globules)
- The nucleus of those fat globules is composed of triglycerides (98 to 99% of the fat in milk)
- Triglyceride = glycerol + fatty acids
- Up to 400 fatty acids in milk



GC analysis of milk fatty acids



Legend

FAMES: Fatty acid methyl esters

C4:0	Butyric acid
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid

Up to 400 fatty acids in milk

Few are mainly present in milk

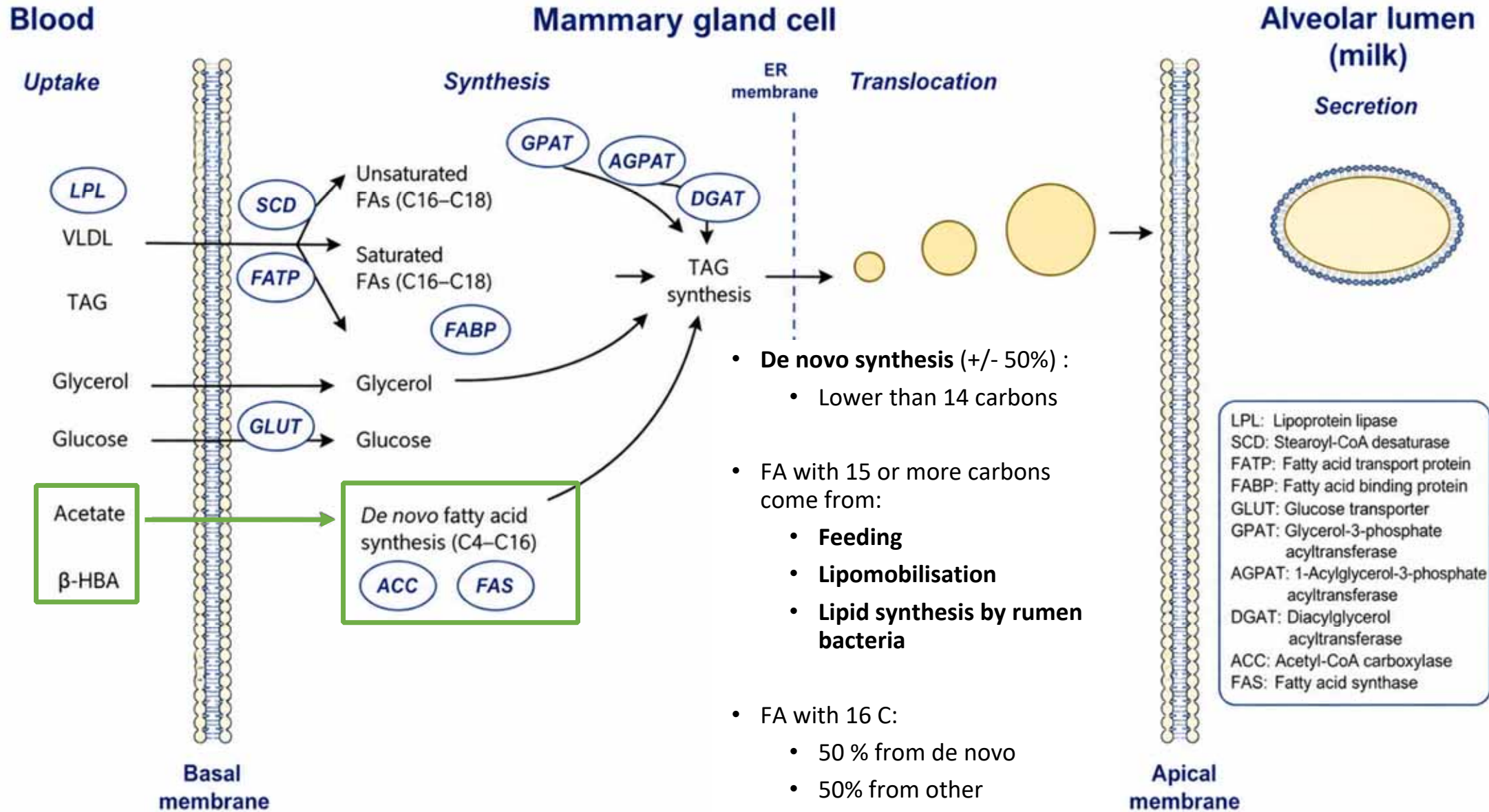
Cow milk

Fatty acid	Name	Range (%)	Central point (%)
C4:0	Butyric	2-5	3.5
C6:0	Caproic	1-5	3
C8:0	Caprylic	1-3	2
C10:0	Capric	2-4	3
C12:0	Lauric	2-5	3.5
C14:0	Myristic	8-14	11
C15:0	Pentadecanoic	1-2	1.5
C16:0	Palmitic	22-35	28.5
C16:1	Palmitoleic	1-3	2
C17:0	Margaric	0.5-1.5	1
C18:0	Steraric	9-14	11.5
C18:1	Oleique	20-30	25
C18:2	Linoleic	1-3	2
C18:3	Linolenic	0.5-2	1.25
TOTAL			98.75

Gervais, 2017, https://www.agrireseau.net/documents/Document_99830.pdf



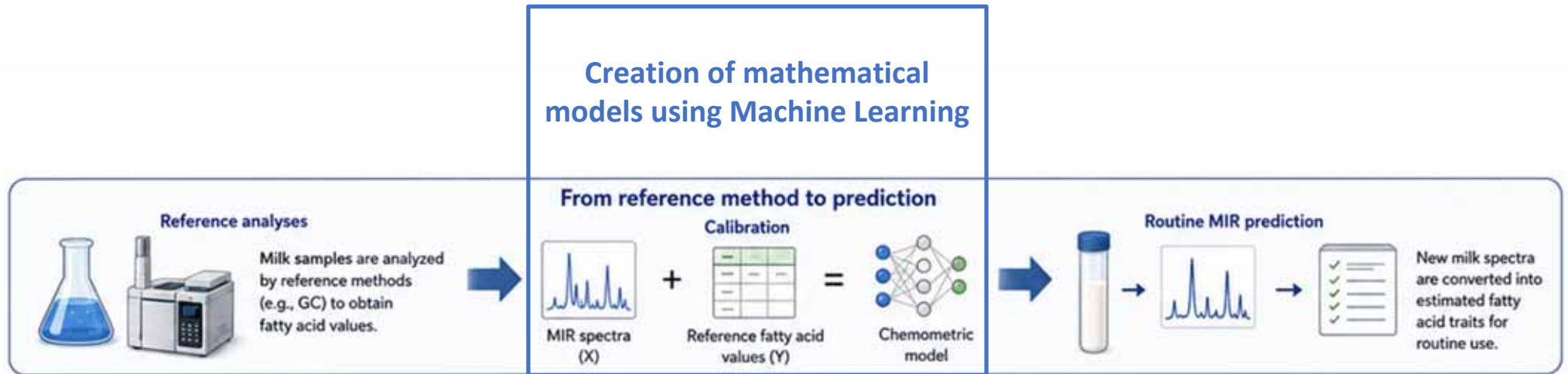
Distinct metabolic pathways for their synthesis by cows



Derived from Gervais, 2017, https://www.agrireseau.net/documents/Document_99830.pdf

Estimation of milk fatty acids using mid-infrared spectrometry

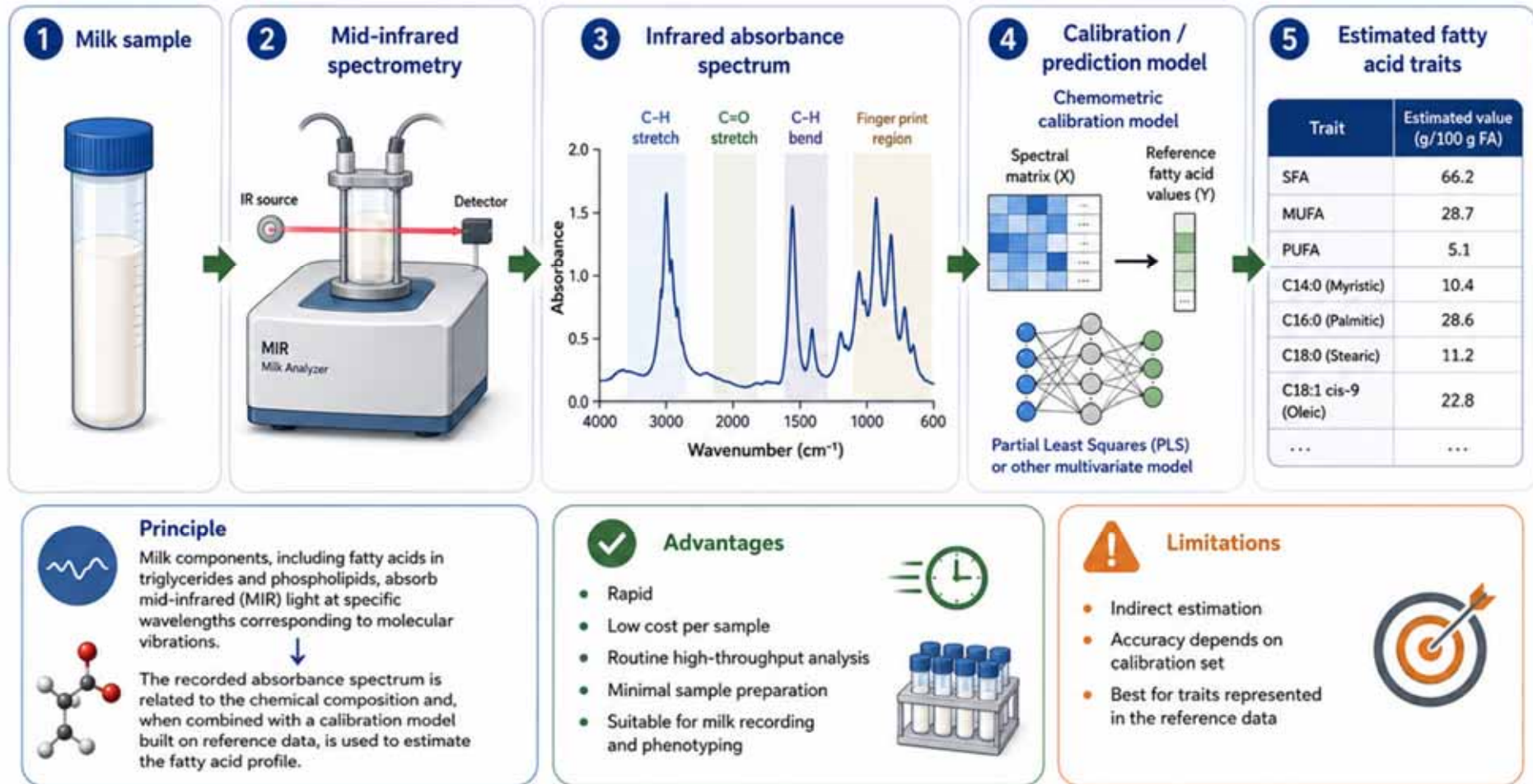
Rapid prediction of fatty acid profile from the milk infrared spectrum



MIR provides indirect estimation of fatty acids and is commonly calibrated against reference analytical methods.

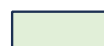
Estimation of milk fatty acids using mid-infrared spectrometry

Rapid prediction of fatty acid profile from the milk infrared spectrum



MIR estimation

Fatty acid	Name	Range (%)	Central point (%)	MIR RMSE (g/dL of milk)	MIR R ² (%)
C4:0	Butyric	2-5	3.5	0.010	89.73
C6:0	Caproic	1-5	3	0.006	91.46
C8:0	Caprylic	1-3	2	0.004	91.30
C10:0	Capric	2-4	3	0.012	89.27
C12:0	Lauric	2-5	3.5	0.014	90.64
C14:0	Myristic	8-14	11	0.035	92.94
C15:0	Pentadecanoic	1-2	1.5	0.007	76.75
C16:0	Palmitic	22-35	28.5	0.096	94.29
C16:1	Palmitoleic	1-3	2	0.016	64.56
C17:0	Margaric	0.5-1.5	1	0.004	72.93
C18:0	Steraric	9-14	11.5	0.060	86.94
C18:1	Oleique	20-30	25	0.072	94.17
C18:2	Linoleic	1-3	2	0.010	80.29
C18:3	Linolenic	0.5-2	1.25	0.005	68.57
TOTAL			98.75		



MIR R² ≥ 90%



75% ≤ MIR R² < 90%

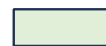


MIR R² < 75%

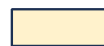
Cabaraux Elisabeth (2026, ICAR presentation)

MIR estimation

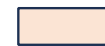
Fatty acid	Name	MIR RMSE	MIR R ²
SFA	Saturated Fatty Acids	0.082	98.88%
UFA	Unsaturated FA	0.080	96.22%
MUFA	Monounsaturated FA	0.072	96.26%
PUFA	Polyunsaturated FA	0.021	82.95%
SCFA	Short chain FA	0.026	93.47%
MCFA	Medium chain FA	0.121	95.98%
LCFA	Long chain FA	0.124	94.97%



MIR R² ≥ 90%



75% ≤ MIR R² < 90%



MIR R² < 75%



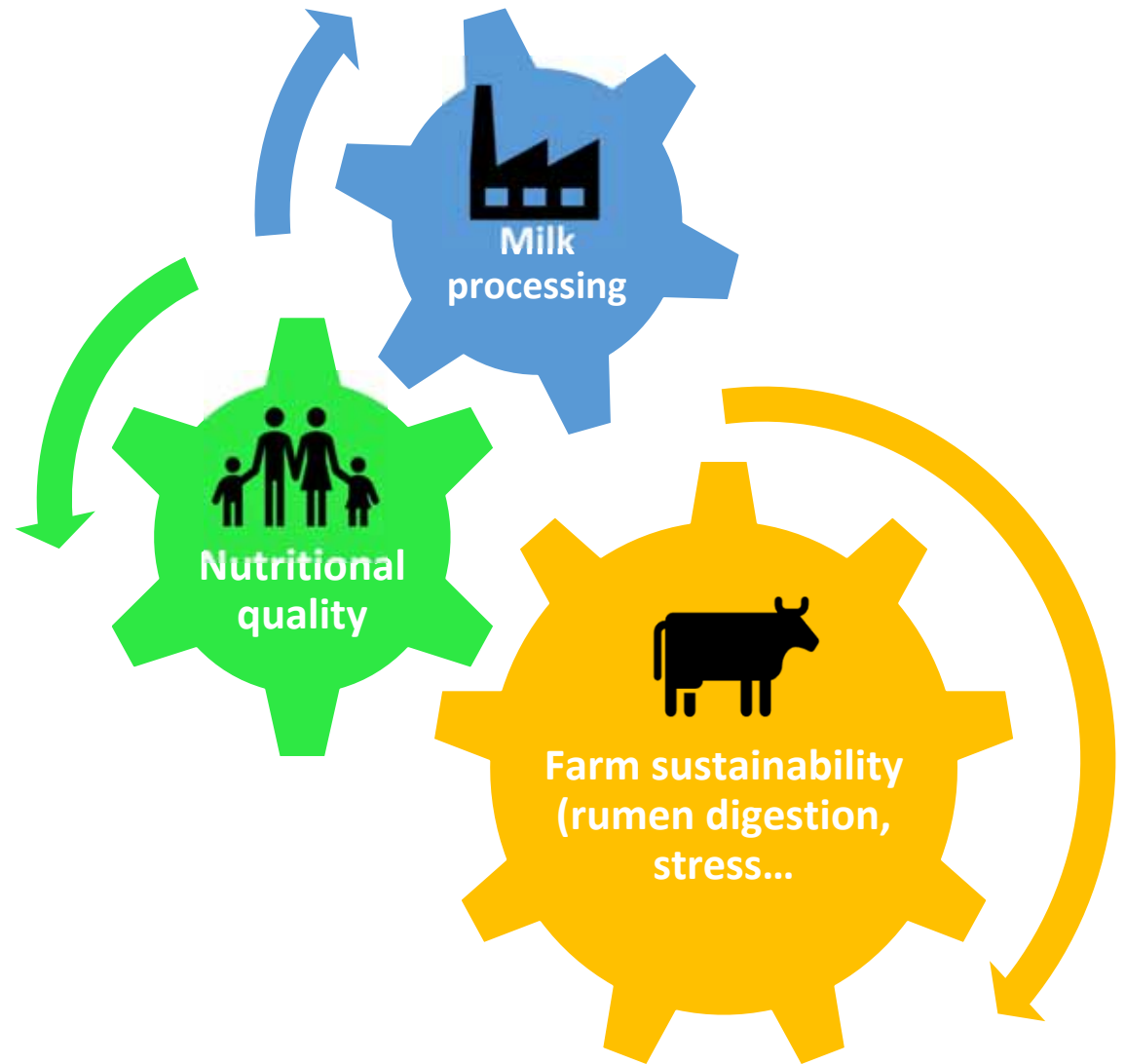
Conclusions

Only few FA are present in milk

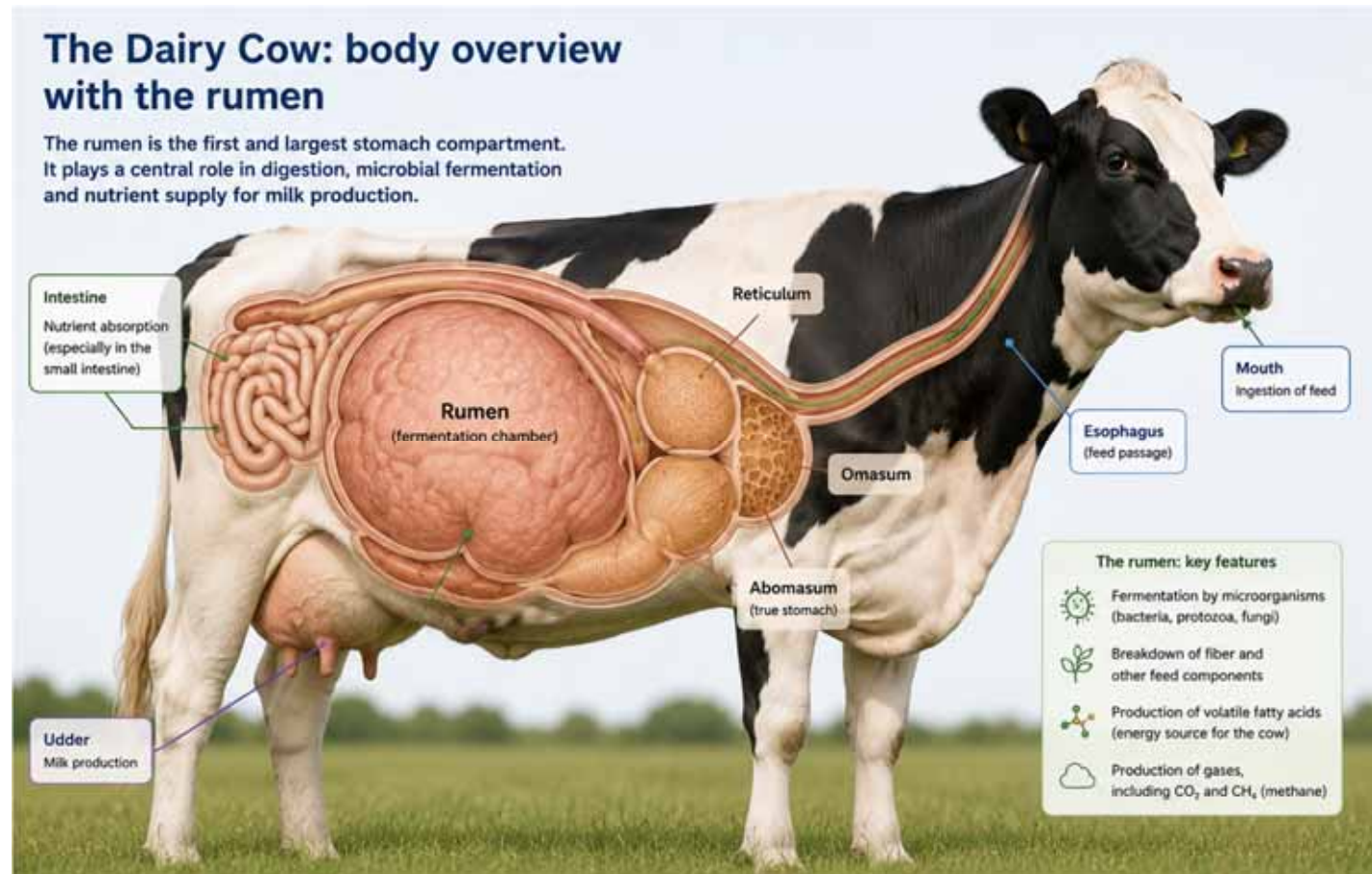
The majority of the most present FA can be predicted with a high accuracy by mid-infrared spectrometry

MIR spectrometry is a low cost analysis compared to GC

Why is it interesting to know the detailed fat composition of milk on a routine basis ?



The fatty acid profile provides information on both **rumen function** and **body reserve mobilization**



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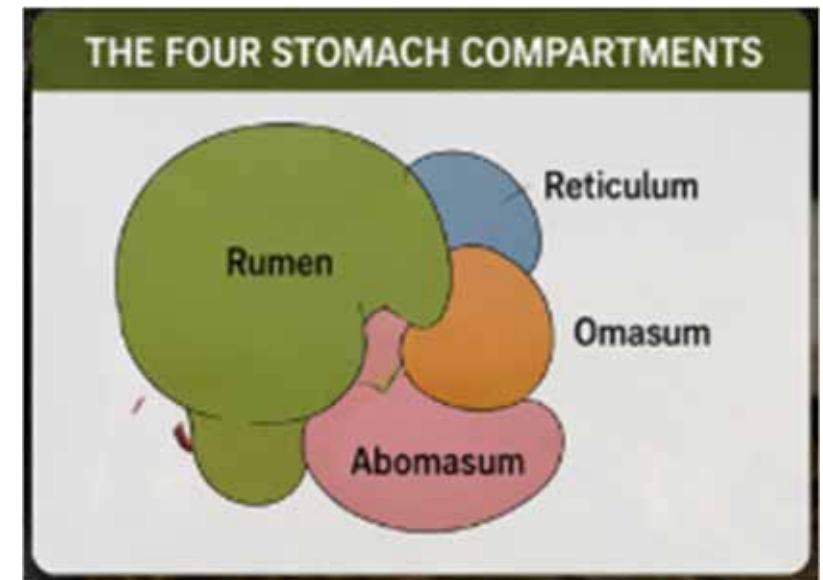
Rumen health

Frequency of data acquisition

- Every 1 to 3 days for each herd delivering milk to the dairy company (**herd level**)
- Every 4 to 6 weeks for each cow in herd joining the milk recording (**cow level**)

Here examples of applications using MIR based fatty acid predictions to monitor herd or cow status:

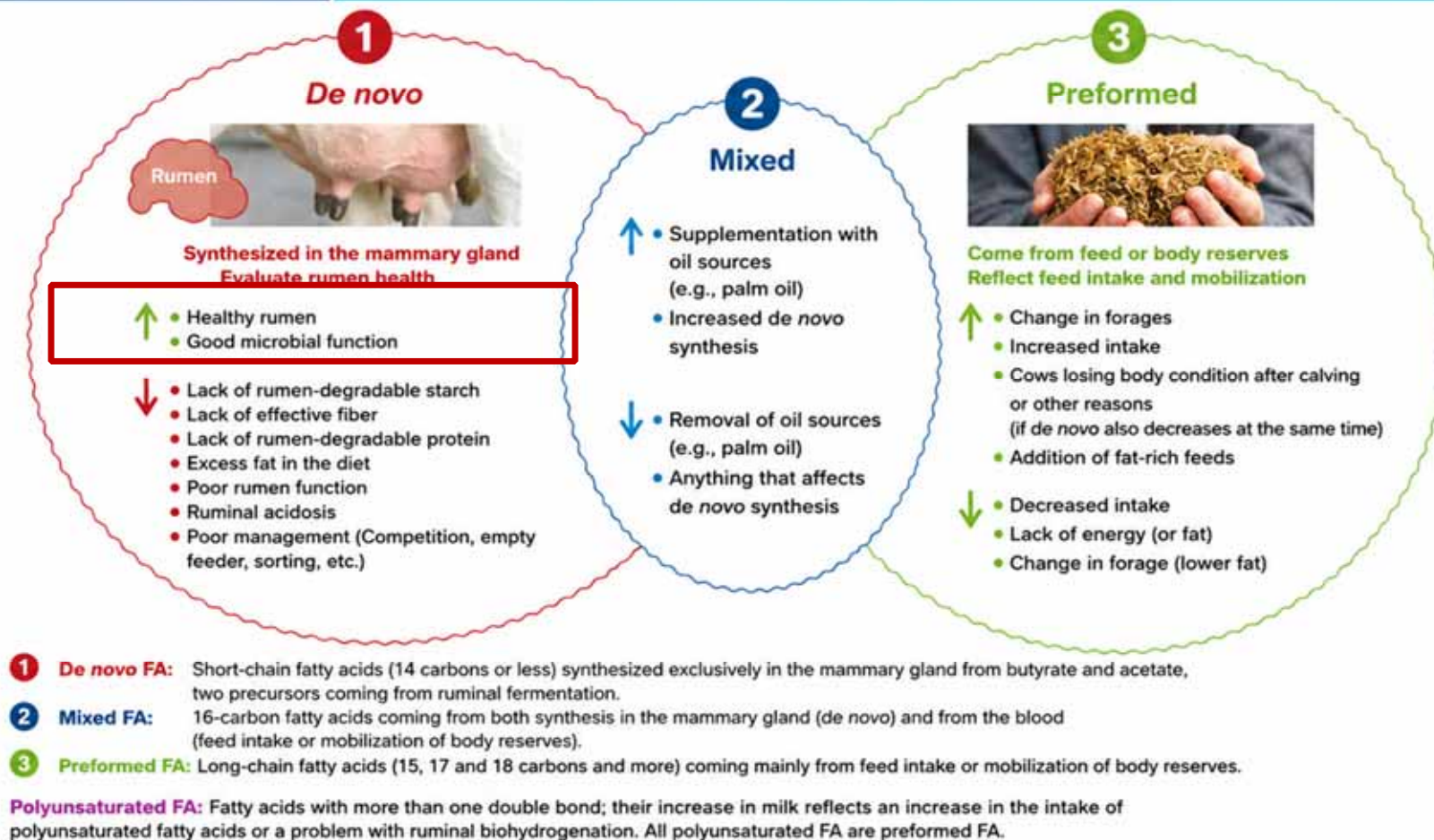
- **Increase of LCFA** in the beginning of lactation → fat body mobilization
- Healthy rumen = **High proportion of De novo FA**



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Cheat Sheet and Solutions

Quick guide to understanding milk fatty acids





de novo (Rumen health)

NOTE

The elements presented here are a few simplified solution ideas. Since every farm is unique, it is strongly recommended to always consult a nutrition advisor and evaluate the economic impact of each change implemented.



HIGH

- The rumen is healthy.
- Is your ration too safe*?
- Could your cows produce more*?

**If you are satisfied with performance, do not change anything.*



AVERAGE

- Results seem stable and around the average.
- Could your cows produce more fat, or more milk*?

**If you are satisfied with performance, do not change anything.*

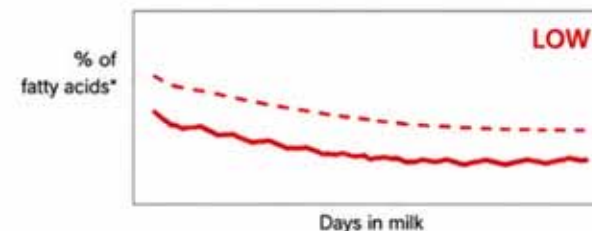
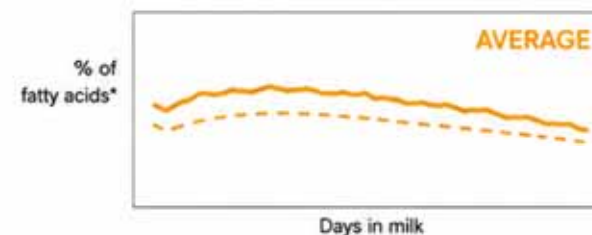
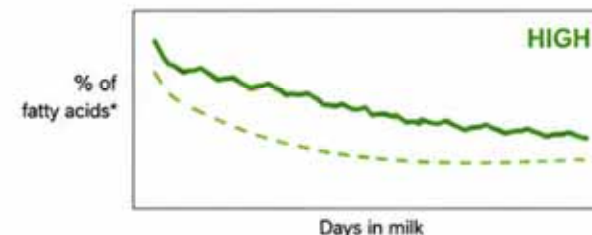


LOW

- Is the rumen functioning properly*?
- Is there a risk of ruminal acidosis or a lack of nutrients**?
- Are your cows eating enough*?
- Validate the ration composition, intake, feeding behavior, cow comfort, rate of passage, genetic potential of cows, etc.

**If you are satisfied with performance, do not change anything.*

****Confirm with milk polyunsaturated fatty acids and milk urea.**



LEGEND

- Dark line – Your herd
- - - Light line – The average

*Fatty acids are expressed on a Milk Fat Basis

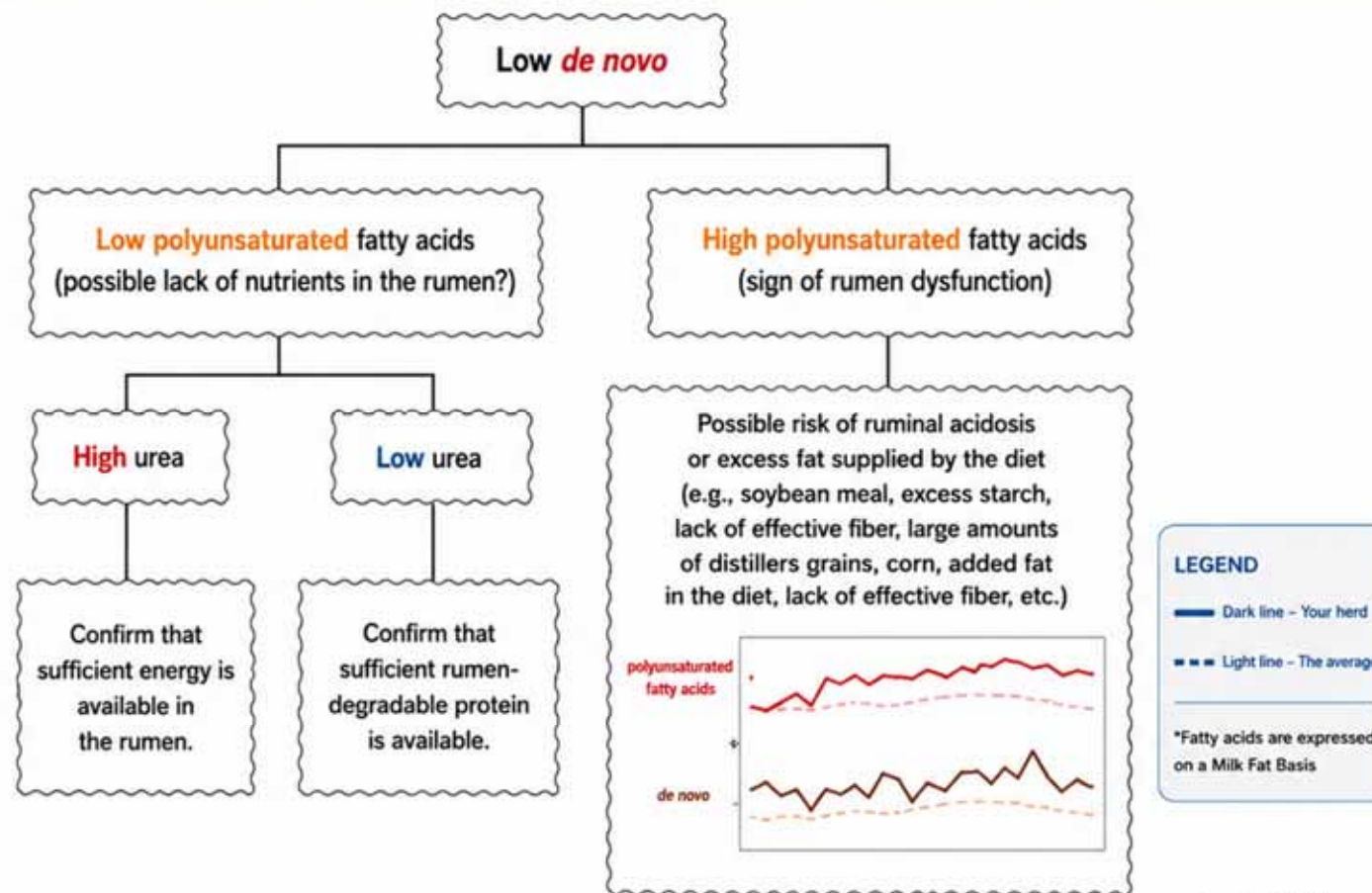
Polyunsaturated fatty acids (Rumen biohydrogenation)

NOTE

The elements presented here are a few simplified solution ideas. Since every farm is unique, it is strongly recommended to always consult a nutrition advisor and evaluate the economic impact of each change implemented.

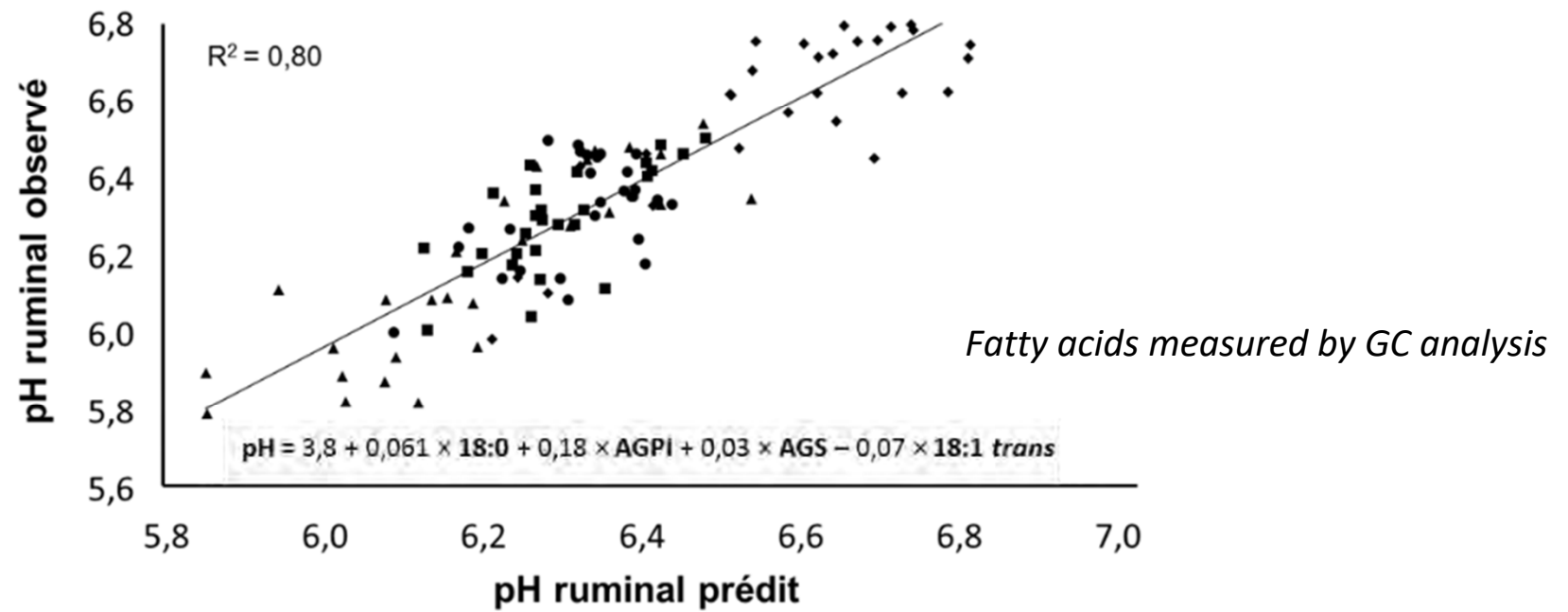
****Pasture-based herds will have high levels of polyunsaturated fatty acids due to the high intake of polyunsaturated fatty acids from the grass consumed.**

If **de novo** are high, the level of **polyunsaturated** fatty acids is usually not critical for rumen health.



November 2021

RUMEN HEALTH

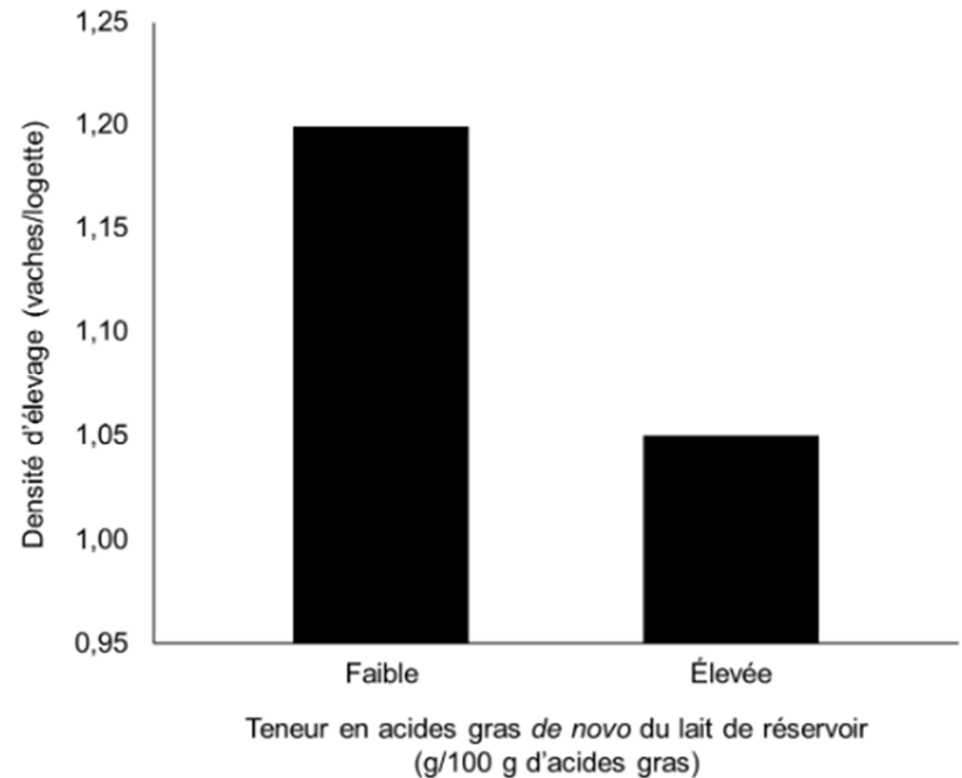


Rumen pH = 3.8 + 0.061 x C18:0 + 0.18 x PUFA + 0.03 x SFA x trans C18:1

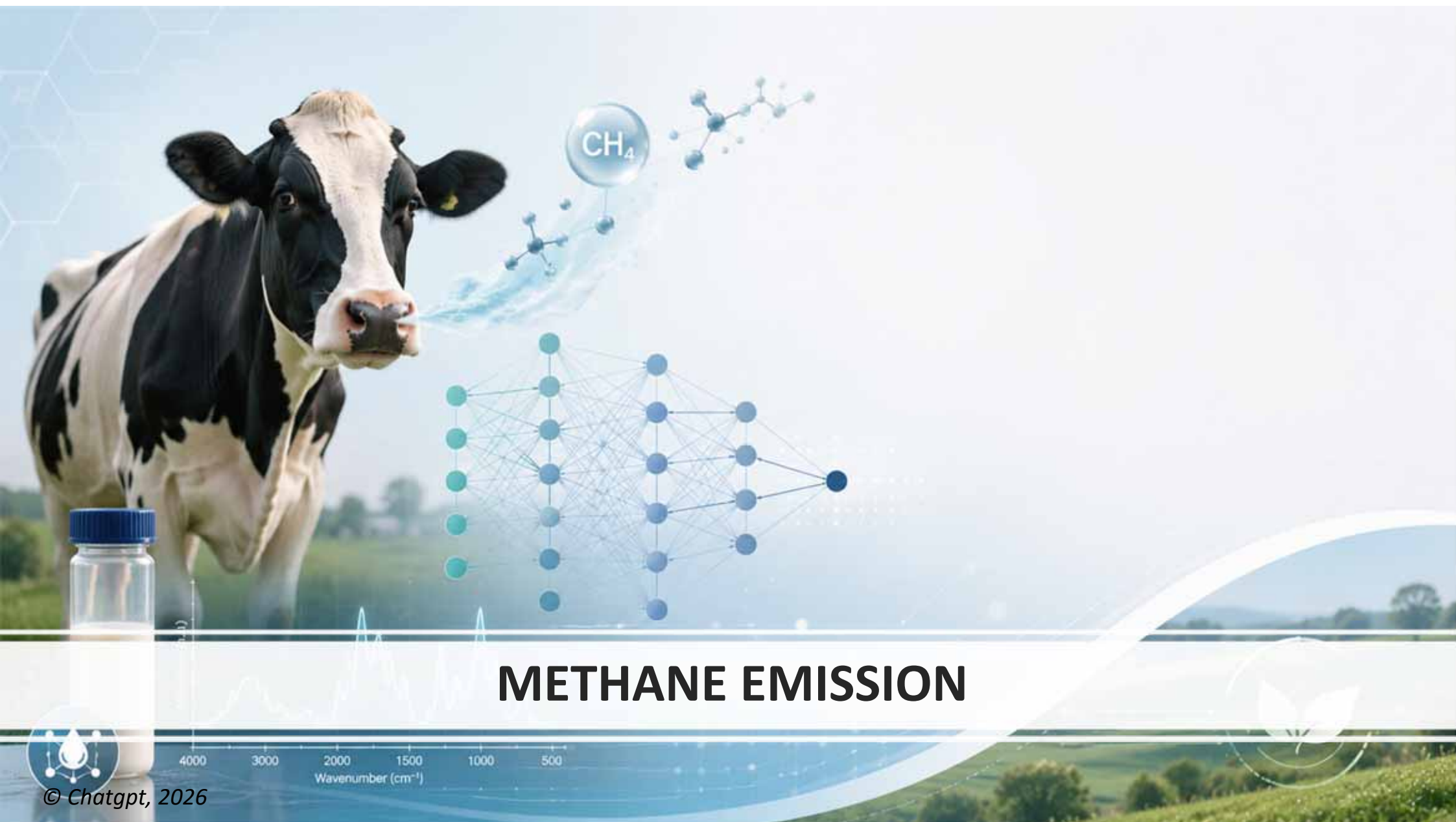
Gervais, 2017, https://www.agrireseau.net/documents/Document_99830.pdf

STOCKING RATE

Following Gervais (2017) **inadequate stocking density**, a limited number of meals and a higher fat content in the ration are factors associated with **low de novo FA levels** in milk as well as reduced secretion of milk components.



Fatty acids measured using GC analysis

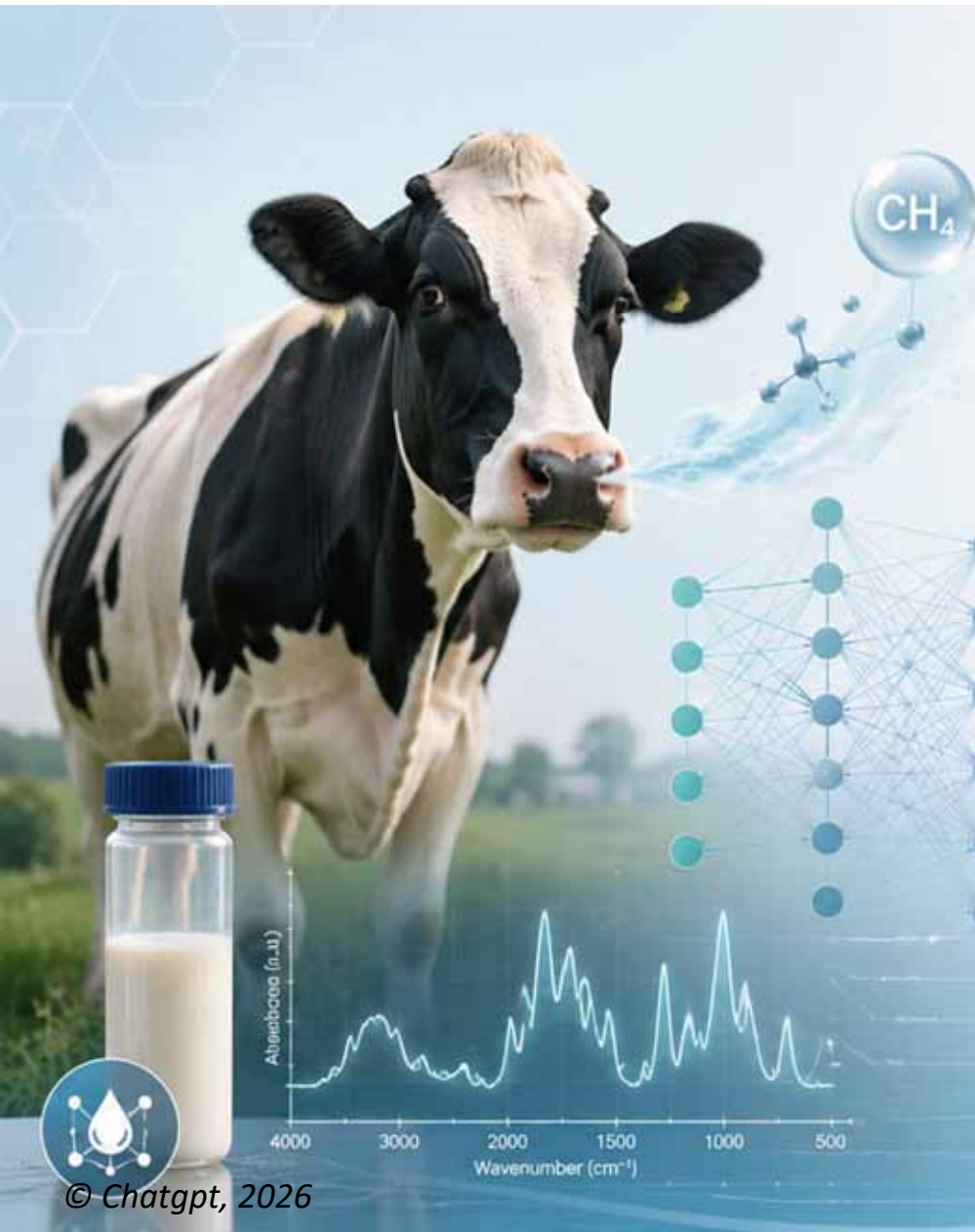


METHANE EMISSIONS

- CH₄ has a global warming potential 28 times that of carbon dioxide.
- The livestock sector represents at a global level 14.5% of human-induced greenhouse gases where emissions of enteric CH₄ contribute to 39%.
- At the cow level, it is also a loss of energy.

Estimation of individual CH₄ emissions from a large number of animals and development of strategies to reduce such emissions represent major challenges for the dairy sector.

Vanlierde et al., 2021, J Sci Food Agric 2021, DOI 10.1002/jsfa.10969

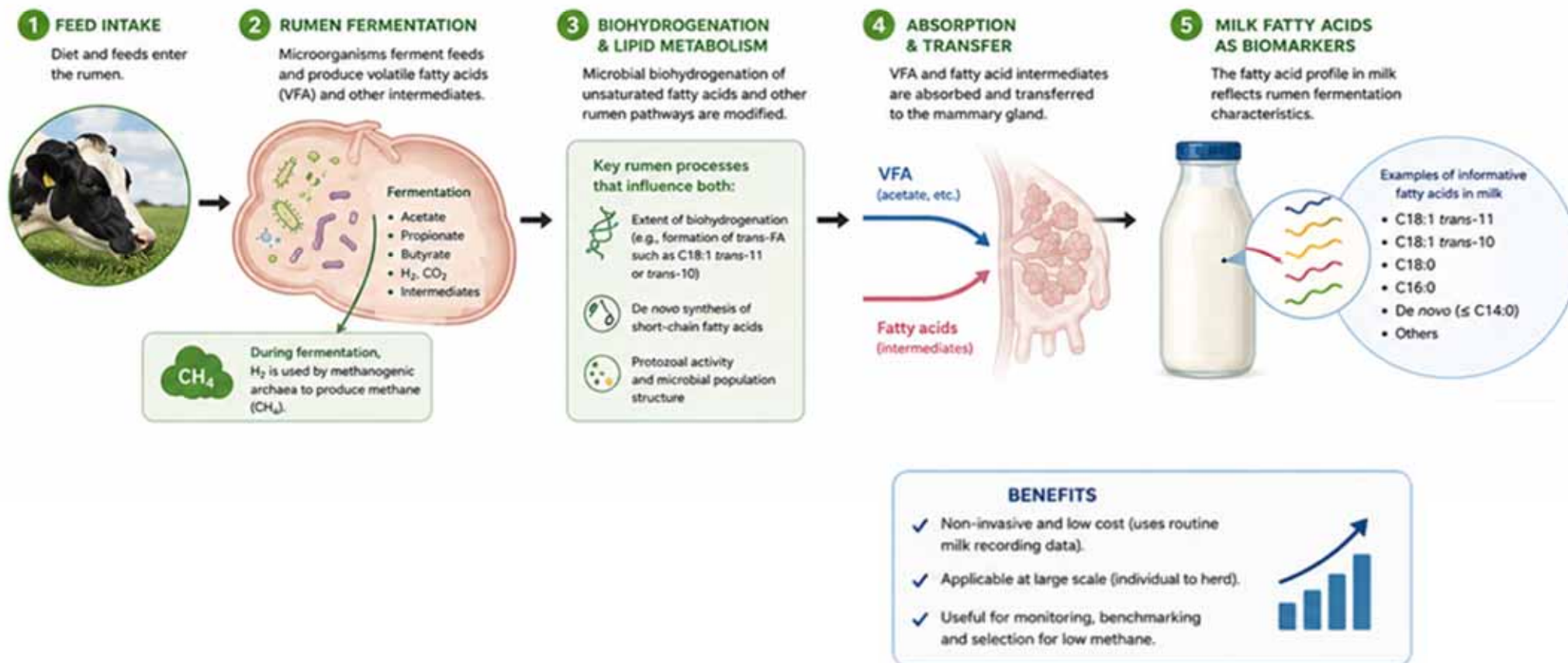


Link with Fatty acids



Key message:

The same rumen processes that drive methane emissions also shape the profile of fatty acids secreted in milk.



Many equations developed

Reference	Equation ¹	Used in current
Methane yield, ⁴ g/kg DMI		
Dijkstra et al., 2011	$CH_4 = 24.6 + 8.74 \times C17:0 \text{ anteiso} - 1.97 \times C18:1 \text{ } t10 + t11 - 9.09 \times C18:1 \text{ } c11 + 5.07 \times C18:1 \text{ } c13$	No ⁵
van Lingen et al., 2014	$CH_4 = 23.39 + 9.74 \times C16:0 \text{ iso} - 1.06 \times C18:1 \text{ } t10 + t11 - 1.75 \times C18:2 \text{ } c9, c12$	Yes
van Engelen et al., 2015	$CH_4 = 28.60 - 1.13 \times C4:0 + 0.36 \times C18:0 - 2.57 \times C18:1 \text{ } t10 + t11 - 9.29 \times C18:1 \text{ } c11$	Yes
	$CH_4 = 27.13 - 3.04 \times C4:0 + 2.71 \times C6:0 - 1.63 \times C18:1 \text{ } t10 + t11$	Yes
Castro-Montoya et al., 2017	$CH_4 = -5.37 - 151 \times C7:0 + 1.21 \times C12:0 + 0.56 \times C16:0 + 13.1 \times C16:1 \text{ } c7$	No ⁶
van Gastelen et al., 2017	$CH_4 = 27.2 - 7.0 \times C18:2 \text{ } c9, t11$	Yes
van Gastelen et al., 2018a	$CH_4 = 28.4 - 18.79 \times C18:1 \text{ } c12 - 5.44 \times C18:3n-3$	Yes
van Gastelen et al., 2018b	$CH_4 = 22.9 + 20.9 \times C15:0 \text{ iso} - 9.6 \times C15:0 \text{ anteiso} + 7.6 \times C17:0 - 2.4 \times C18:1 \text{ } t11 - 2.7 \times C18:1 \text{ } t15, c11 - 4.4 \times C18:3n-3$	Yes
Bougouin et al., 2019	$CH_4 = 21.0 + 10.5 \times C16:0 \text{ iso} - 2.7 \times C18:1 \text{ } c11 - 0.8 \times C18:1 \text{ } t10 - 1.1 \times C18:2 \text{ } c9, c12$	Yes
Methane intensity, g/kg CM		
van Lingen et al., 2014	$CH_4 = 21.13 - 1.38 \times C4:0 + 8.53 \times C16:0 \text{ iso} - 0.22 \times C18:1 \text{ } c9 - 0.59 \times C18:1 \text{ } t10 + t11$	Yes
Castro-Montoya et al., 2017 ⁸	$CH_4 = -122 + 20.1 \times C4:0 + 2.355 \times C7:0 + 4.74 \times C16:0 + 64.9 \times C17:1 \text{ } c9 - 53.8 \times C18:1 \text{ } c13 + 19.4 \times C22:6n-3 + 435 \times C4:0$ $\times C7:0 - 0.91 \times C4:0 \times C16:0 - 87.0 \times C7:0 \times C16:0 - 3,349 \times C7:0 \times 17:1 \text{ } c9$	No ⁹
van Gastelen et al., 2017	$CH_4 = 16.5 + 24.6 \times C15:0 \text{ iso} - 15.5 \times C17:0 + 52.4 \times C22:0$	Yes
van Gastelen et al., 2018a	$CH_4 = 13.7 - 1.33 \times C4:0 + 32.00 \times C15:0 \text{ iso} - 4.12 \times C18:3n-3 - 35.44 \times C20:1 \text{ } c11 + 27.62 \times C22:5n-3$	No ¹⁰
van Gastelen et al., 2018b	$CH_4 = 8.0 + 24.8 \times C15:0 \text{ iso} + 10.3 \times C17:0 \text{ iso} - 6.6 \times 18:1 \text{ } t15, c11 + 22.7 \times C22:5n-3$	Yes
Bougouin et al., 2019 ⁸	$CH_4 = 13.8 + 9.8 \times C16:0 \text{ iso} - 2.4 \times C18:1 \text{ } c15 - 0.4 \times C18:1 \text{ } t10 + t11$	No ¹¹
	$CH_4 = 15.3 + 3.9 \times C16:0 \text{ iso} - 0.5 \times C18:1 \text{ } t10 + t11 + 1.9 \times \text{milk fat} + 2.2 \times \text{milk protein} - 3.2 \times \text{milk lactose}$	Yes
Daily methane production, ¹² g/d		
Mohammed et al., 2011	$CH_4 = 272.4 - 486.2 \times C17:1 \text{ } c9 - 122.7 \times C18:1 \text{ } c11 + 2,220 \times \text{CLA } t,t - 11.76 \times \sum C18:1 \text{ } t + 260.1 \times C15:0 \text{ anteiso}$	No ¹³
Williams et al., 2014	$CH_4 = 225 + 149.8 \times C8:0$	Yes
	$CH_4 = 629 - 6.03 \times \sum C18$	Yes
	$CH_4 = 539 + 50.8 \times C8:0 - 5.26 \times \sum C18$	Yes
Rico et al., 2016	$CH_4 = 669.1 + 838.7 \times C14:1 \text{ } c11 - 493.2 \times C17:1 \text{ } c9 - 44.2 \times C18:1 \text{ } c11 - 963.7 \times C18:2 \text{ } t8, c13$	No ¹⁴
Castro-Montoya et al., 2017	$CH_4 = 471 - 137 \times C16:1 \text{ } c13 - 824 \times C16:1 \text{ } t14 + 138 \times C18:1 \text{ } t10 - 280 \times C18:1 \text{ } t12 - 325 \times C18:2 \text{ } t11, c15$	No ¹⁵
van Gastelen et al., 2017	$CH_4 = 211.2 + 50.4 \times C4:0 + 77.7 \times C14:1 \text{ } c9 - 82.0 \times C18:1 \text{ } t11$	Yes
Engelke et al., 2018 ¹⁶	$CH_4 = -2,534 + 33.8 \times \text{SFA} + 55.0 \times C18:0 + 30.9 \times C18:1 \text{ } t$	Yes
	$CH_4 = -1,364 + 9.58 \times \text{ECM} + 18.5 \times \text{SFA} + 32.4 \times C18:0$	Yes
van Gastelen et al., 2018a	$CH_4 = 522 - 98.0 \times C18:1 \text{ } t15 + C18:1 \text{ } c11 - 116.7 \times C18:2 \text{ } c9, t11 - 80.9 \times C18:3n-3$	Yes
van Gastelen et al., 2018b	$CH_4 = 507.9 + 62.9 \times C15:0 - 240.6 \times C17:1 \text{ } c9 - 202.8 \times C18:1 \text{ } t10 - 59.3 \times C18:1 \text{ } t11 + 48.1 \times C18:2n-6 - 187.1 \times C18:3n-3 +$ $326.4 \times C20:4n-3 - 816.8 \times C24:0$	No ¹⁷
Bougouin et al., 2019	$CH_4 = 467.3 + 26.7 \times C10:0 - 101.1 \times (C17:0 \text{ iso} + C16:1 \text{ } t9) - 122.8 \times C18:1 \text{ } c11 - 37.3 \times C18:2 \text{ } t11, c15$	Yes
	$CH_4 = 372.5 + 18.6 \times C10:0 - 79.3 \times C17:0 \text{ iso} + C16:1 \text{ } t9 - 103.4 \times C18:1 \text{ } c11 - 38.9 \times C18:2 \text{ } t11, c15 + 3.6 \times \text{milk yield}$	Yes
	$CH_4 = 178.6 + 22.7 \times C10:0 - 100 \times C18:1 \text{ } c11 - 41.5 \times C18:2 \text{ } t11, c15 + 4.2 \times \text{milk yield} + 31.2 \times \text{milk fat}$	Yes

Massaro et al., 2024, J. Dairy Sci. <https://doi.org/10.3168/jds.2024-24814>

Using MIR-based traits ?

Dataset from Vanlierde et al. (2021)	N	Status	R ² (%) N=1089	RMSE (g/day) N=1,089
$467.3 + 26.7 * \text{acides\$pred_C10} - 101.1 * (\text{acides\$pred_C17} + \text{acides\$pred_C16_1c}) - 122.8 * \text{acides\$pred_C18_1cis11} - 37.3 * \text{acides\$pred_C18_2c9t11}$	Bougouin , 2019	PARTIAL	8.82	109.13
$372.5 + 18.6 * \text{acides\$pred_C10} - 79.3 * (\text{acides\$pred_C17} + \text{acides\$pred_C16_1c}) - 103.4 * \text{acides\$pred_C18_1cis11} - 38.9 * \text{acides\$pred_C18_2c9t11} + 3.6 * \text{acides\$Lait_sm}$	Bougouin , 2019	PARTIAL	8.48	108.36
$272.4 - 486.2 * \text{acides\$pred_C17_1} - 122.7 * \text{acides\$pred_C18_1cis11} + 2.220 * \text{acides\$pred_C18_2c9t11} - 11.76 * \text{acides\$pred_totC18_1trans} + 260.1 * \text{acides\$pred_C15}$	Mohamm ed	COMPLETE	1.21	169.63
$225 + 149.8 * (\text{acides\$pred_C8} * (100 / \text{acides\$Fat}))$	Williams, 2014	COMPLETE	10.80	98.77
$629 - 6.03 * ((\text{acides\$pred_C18} + \text{acides\$pred_totC18_1} + \text{acides\$pred_C18_2} + \text{acides\$pred_C18_3c9c12c15}) * (100 / \text{acides\$Fat}))$	Williams, 2014	COMPLETE	16.80	95.11
$539 + 50.8 * (\text{acides\$pred_C8} * (100 / \text{acides\$Fat})) - 5.26 * ((\text{acides\$pred_C18} + \text{acides\$pred_totC18_1} + \text{acides\$pred_C18_2} + \text{acides\$pred_C18_3c9c12c15}) * (100 / \text{acides\$Fat}))$	Williams, 2014	COMPLETE	16.47	96.23
$669.1 + 838.7 * \text{acides\$pred_C14_1} - 493.2 * \text{acides\$pred_C17_1} - 44.2 * \text{acides\$pred_C18_1cis11} - 963.7 * \text{acides\$pred_C18_2c9t11}$	Rico, 2016	PARTIAL	11.39	274.97
$211.2 + 50.4 * (\text{acides\$pred_C4} * (100 / \text{acides\$Fat})) + 77.7 * (\text{acides\$pred_C14_1} * (100 / \text{acides\$Fat})) - 82.0 * (\text{acides\$pred_C18_1c13,14t16} * (100 / \text{acides\$Fat}))$	Van Gastelen, 2017	COMPLETE	14.57	99.08
$-2534 + 33.8 * (\text{acides\$pred_SAT} * (100 / \text{acides\$Fat})) + 55.0 * (\text{acides\$pred_C18} * (100 / \text{acides\$Fat})) + 30.9 * (\text{acides\$pred_totC18_1trans} * (100 / \text{acides\$Fat}))$	Engelke, 2018	COMPLETE	11.54	163.62

Using MIR-based fatty acids

- Data set coming from Vanlierde et al. (2021)
- Data split:
 - 80% for training (N=866)
 - 20% for test (N=223)
- 4 different sets of features
 - Using animal characteristics + milk yield + MIR-based fatty acids
 - Using MIR-based fatty acids
 - Using only spectral data
 - Using spectral data + animal characteristics + milk yield
- 3 different types of model:
 - Penalized linear regression : LASSO
 - Partial Least Squares regression : PLS
 - Random Forest : RF

Vanlierde et al., 2021, J Sci Food Agric 2021, DOI 10.1002/jsfa.10969

Using MIR-based fatty acids

Features	Model	Hyperparameter	RMSE_cv (g/day) (N=866)	R ² _cv (%) (N=866)	RMSE_test (g/day) (N=223)
FA + animal	PLS	Ncomp = 30	69.30	55.53	77.62
	LASSO	Lambda= 0.1072	69.08	55.60	75.76
	RF	Mtry=25	60.27	66.89	74.02
FA	PLS	Ncomp=27	70.84	53.49	79.63
	LASSO	Lambda=0.0811	70.70	53.53	78.83
	RF	Mtry = 20	64.07	62.64	74.59
Spectrum	PLS	Ncomp=18	70.61	54.76	69.36
	LASSO	Lambda=0.4329	69.43	55.23	70.70
	RF	Mtry = 30	68.28	58.68	81.02
Spectrum + animal	PLS	Ncomp=18	69.63	55.94	67.31
	LASSO		68.01	56.65	67.65

Adding information of animal like days in milk, parity and milk yield improves the prediction → same conclusion as Vanlierde et al. (2021)

Vanlierde et al., 2021, J Sci Food Agric 2021, DOI 10.1002/jsfa.10969

Using the spectral data instead of only the fatty acids improves the prediction → same conclusion as Dehareng et al. (2012)

Dehareng et al., 2012, Animal , <https://doi.org/10.1017/S1751731112000456>

Using MIR-based fatty acids

Features	Model	RMSE_test (g/day) (N=223)
FA + animal	PLS	77.62
	LASSO	75.76
	RF	74.02
FA	PLS	79.63
	LASSO	78.83
	RF	74.59
Spectrum	PLS	69.36
	LASSO	70.70
	RF	81.02
Spectrum + animal	PLS	67.31
	LASSO	67.65



Direct use on bulk tank milk



Prediction accuracy is lower !

Using MIR-based fatty acids

Features	Model	RMSE_test (g/day) (N=223)
FA + animal	PLS	77.62
	LASSO	75.76
	RF	74.02
FA	PLS	79.63
	LASSO	78.83
	RF	74.59
Spectrum	PLS	69.36
	LASSO	70.70
	RF	81.02
Spectrum + animal	PLS	67.31
	LASSO	67.65



Better accuracy with animal characteristics



Association between milk recording and dairy industry



Many equations developed

Reference	Equation ¹	Used in current
Methane yield, ⁴ g/kg DMI		
Dijkstra et al., 2011	$CH_4 = 24.6 + 8.74 \times C17:0 \text{ anteiso} - 1.97 \times C18:1 \text{ r10} + \text{r11} - 9.09 \times C18:1 \text{ c11} + 5.07 \times C18:1 \text{ c13}$	No ⁵
van Lingen et al., 2014	$CH_4 = 23.39 + 9.74 \times C16:0 \text{ iso} - 1.06 \times C18:1 \text{ r10} + \text{r11} - 1.75 \times C18:2 \text{ c9,c12}$	Yes
van Engelen et al., 2015	$CH_4 = 28.60 - 1.13 \times C4:0 + 0.36 \times C18:0 - 2.57 \times C18:1 \text{ r10} + \text{r11} - 9.29 \times C18:1 \text{ c11}$	Yes
	$CH_4 = 27.13 - 3.04 \times C4:0 + 2.71 \times C6:0 - 1.63 \times C18:1 \text{ r10} + \text{r11}$	Yes
Castro-Montoya et al., 2017	$CH_4 = -5.37 - 151 \times C7:0 + 1.21 \times C12:0 + 0.56 \times C16:0 + 13.1 \times C16:1 \text{ c7}$	No ⁶
van Gastelen et al., 2017	$CH_4 = 27.2 - 7.0 \times C18:2 \text{ c9,r11}$	Yes
van Gastelen et al., 2018a	$CH_4 = 28.4 - 18.79 \times C18:1 \text{ c12} - 5.44 \times C18:3n-3$	Yes
van Gastelen et al., 2018b	$CH_4 = 22.9 + 20.9 \times C15:0 \text{ iso} - 9.6 \times C15:0 \text{ anteiso} + 7.6 \times C17:0 - 2.4 \times C18:1 \text{ r11} - 2.7 \times C18:1 \text{ r15,c11} - 4.4 \times C18:3n-3$	Yes
Bougouin et al., 2019	$CH_4 = 21.0 + 10.5 \times C16:0 \text{ iso} - 2.7 \times C18:1 \text{ c11} - 0.8 \times C18:1 \text{ r10} - 1.1 \times C18:2 \text{ c9,c12}$	Yes
Methane intensity, ⁷ g/kg CM		
van Lingen et al., 2014	$CH_4 = 21.13 - 1.38 \times C4:0 + 8.53 \times C16:0 \text{ iso} - 0.22 \times C18:1 \text{ c9} - 0.59 \times C18:1 \text{ r10} + \text{r11}$	Yes
If those equations are correct using GC data, this could be used on the fatty acid database constructed to create FA equations... This could enlarge the dataset... 		
Rico et al., 2016	$CH_4 = 539 + 50.8 \times C8:0 - 5.26 \times \sum C18$	Yes
Castro-Montoya et al., 2017	$CH_4 = 669.1 + 838.7 \times C14:1 \text{ c11} - 493.2 \times C17:1 \text{ c9} - 44.2 \times C18:1 \text{ c11} - 963.7 \times C18:2 \text{ r8,c13}$	No ¹⁴
van Gastelen et al., 2017	$CH_4 = 471 - 137 \times C16:1 \text{ c13} - 824 \times C16:1 \text{ r14} + 138 \times C18:1 \text{ r10} - 280 \times C18:1 \text{ r12} - 325 \times C18:2 \text{ r11,c15}$	No ¹⁵
Engelke et al., 2018 ¹⁶	$CH_4 = 211.2 + 50.4 \times C4:0 + 77.7 \times C14:1 \text{ c9} - 82.0 \times C18:1 \text{ r11}$	Yes
	$CH_4 = -2,534 + 33.8 \times SFA + 55.0 \times C18:0 + 30.9 \times C18:1 \text{ r}$	Yes
	$CH_4 = -1,364 + 9.58 \times ECM + 18.5 \times SFA + 32.4 \times C18:0$	Yes
van Gastelen et al., 2018a	$CH_4 = 522 - 98.0 \times C18:1 \text{ r15} + C18:1 \text{ c11} - 116.7 \times C18:2 \text{ c9,r11} - 80.9 \times C18:3n-3$	Yes
van Gastelen et al., 2018b	$CH_4 = 507.9 + 62.9 \times C15:0 - 240.6 \times C17:1 \text{ c9} - 202.8 \times C18:1 \text{ r10} - 59.3 \times C18:1 \text{ r11} + 48.1 \times C18:2n-6 - 187.1 \times C18:3n-3 + 326.4 \times C20:4n-3 - 816.8 \times C24:0$	No ¹⁷
Bougouin et al., 2019	$CH_4 = 467.3 + 26.7 \times C10:0 - 101.1 \times (C17:0 \text{ iso} + C16:1 \text{ r9}) - 122.8 \times C18:1 \text{ c11} - 37.3 \times C18:2 \text{ r11,c15}$	Yes
	$CH_4 = 372.5 + 18.6 \times C10:0 - 79.3 \times C17:0 \text{ iso} + C16:1 \text{ r9} - 103.4 \times C18:1 \text{ c11} - 38.9 \times C18:2 \text{ r11,c15} + 3.6 \times \text{milk yield}$	Yes
	$CH_4 = 178.6 + 22.7 \times C10:0 - 100 \times C18:1 \text{ c11} - 41.5 \times C18:2 \text{ r11,c15} + 4.2 \times \text{milk yield} + 31.2 \times \text{milk fat}$	Yes

Massaro et al., 2024, J. Dairy Sci. <https://doi.org/10.3168/jds.2024-24814>



Milk processing



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Grassland

- Natural barrier against desertification through its root structure and soil cover
- Fire prevention and management by limiting the amount of fuel present
- Relatively stable carbon sink
- Average net annual productivity for the biome of 161 g of carbon per year per m² over a 5-year study
- Access to a lower-cost feed resource which requires minimal supplementation for dairy cows
- Many important nutrients



Farm and industry interest to promote a sustainable dairy farming

*Soyeurt et al., Animals 2022, 12(19), 2663;
<https://doi.org/10.3390/ani12192663>*

Detection of Grass-Based diet

	TenFold Stratified Cross-Validation				Validation
	N	AUC ¹	Sensitivity	Specificity	Accuracy
GRASS1	533,786	94.71 ± 0.07	86.78 ± 0.17	88.61 ± 0.17	89.66
GRASS2	397,409	96.21 ± 0.08	88.41 ± 0.27	90.84 ± 0.19	90.95
GRASS3	265,876	97.43 ± 0.08	90.55 ± 0.18	92.99 ± 0.21	91.40

Soyeurt et al., Animals 2022, 12(19), 2663; <https://doi.org/10.3390/ani12192663>

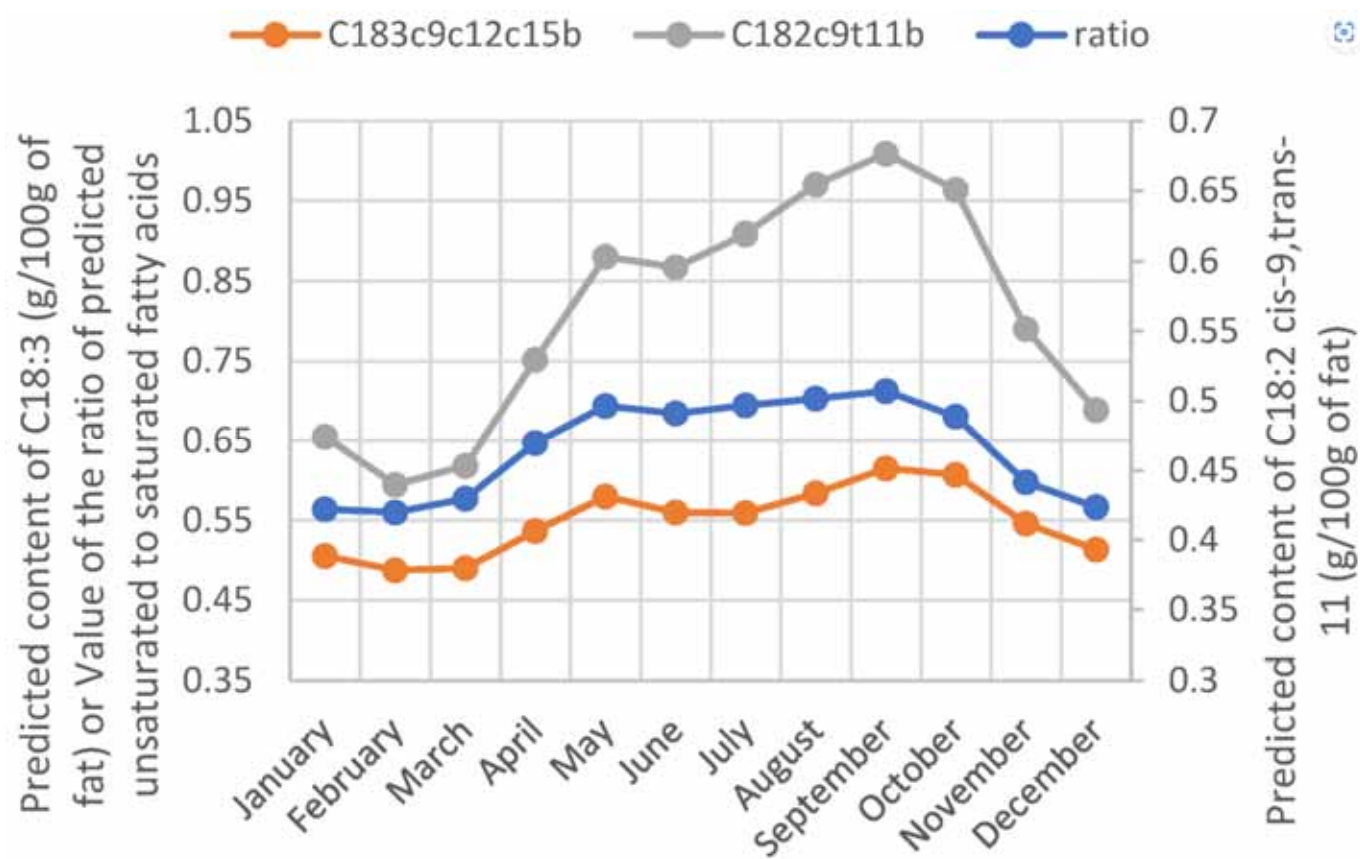
Predicted pasture proportion in the cows' diets validation **R² of 0.81** and Standard error of prediction (SEP) of **11.7% dry matter**.

Coppa et al., J. Dairy Sci., 2021, <https://doi.org/10.3168/jds.2020-18468>

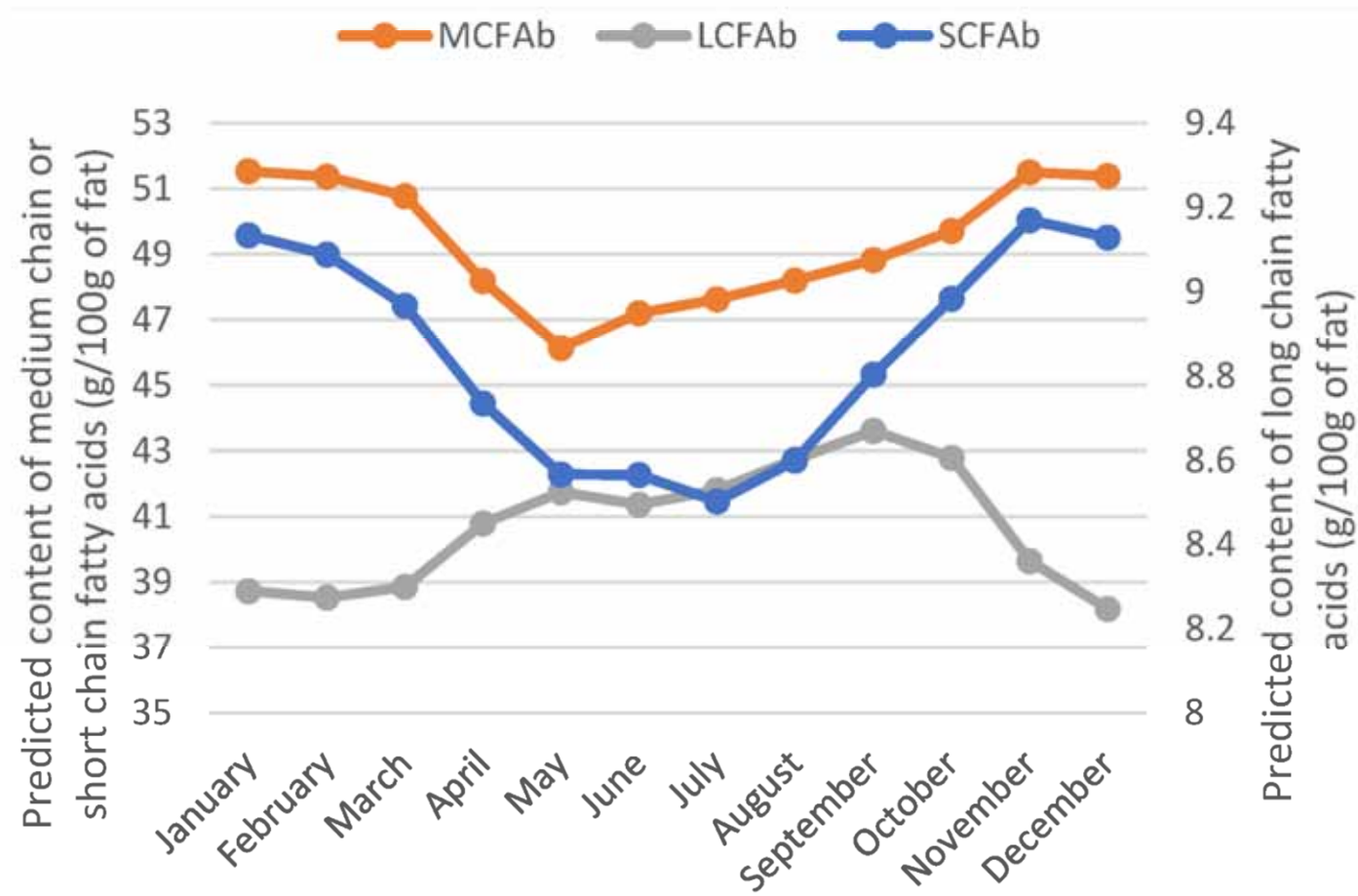
The probability of belonging to the herbage group : R² of **0.93** with a sensitivity of 0.94, and a specificity of 0.93. Predicting the percentage of herbage in the diet with an error of **8.77%**.

Dichou et al., J. Dairy Sci., 2025, <https://doi.org/10.3168/jds.2025-26794>

Real fingerprint in MIR information



Real fingerprint in MIR information



Feed authentication

- Authentication of the cow feeding restrictions included in the specification of 2 Protected Designation of Origin (PDO) cheeses (Cantal and Laguiole)
- Models built on the simultaneous respect of all the criteria of the feeding restrictions of PDO cheese specifications achieved an accuracy, specificity, sensitivity, and **precision >90%**.



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Coppa et al., J. Dairy Sci., 2021, <https://doi.org/10.3168/jds.2020-18468>



Society level

Milk is one of the most consumed food in the world

Interest to promote high nutritional quality food

No.	Index	Full Name	Calculation Formula	Application
1	PUFA/SFA	Polyunsaturated fatty acid/saturated fatty acid ratio	$\Sigma\text{PUFA}/\Sigma\text{SFA}$	Seaweeds [27–29], crops [30,31], plant oil [32,33], shellfish [34], fish [34–40], meat [41–53], and dairy products [54–57]
2	IA	Index of atherogenicity	$[\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}]/\Sigma\text{UFA}$	Seaweeds [27–29,58,59], crops [30,31,60,61], plant oil [33,62], shellfish [63], shrimp [64], fish [36–39,65–73], meat [41–43,48–50,52,53,74–77], and dairy products [54–56,78–89]
3	IT	Index of thrombogenicity	$(\text{C14:0} + \text{C16:0} + \text{C18:0})/[(0.5 \times \Sigma\text{MUFA}) + (0.5 \times \Sigma n-6 \text{ PUFA}) + (3 \times \Sigma n-3 \text{ PUFA}) + (n-3/n-6)]$	Seaweeds [27–29,58,59], crops [30,31,60,61], plant oil [62], shellfish [63], shrimp [64], fish [36–39,65–68,70–73], meat [43,48,50,52,53,75,77], and dairy products [54,55,78,80,86–89]
4	HH	Hypocholesterolemic /hypercholesterolemic ratio	$(\text{cis-C18:1} + \Sigma\text{PUFA})/(\text{C12:0} + \text{C14:0} + \text{C16:0})$	Seaweeds [90], plant oil [62], shellfish [34], fish [34,36–39,72], meat [46–48,52,77,91], and dairy products [54,55,78,86,87]
5	HPI	Health-promoting index	$\Sigma\text{UFA}/[\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}]$	Milk [92–94] and cheese [57,94,95]
6	UI	Unsaturation index	$1 \times (\% \text{ monoenoics}) + 2 \times (\% \text{ dienoics}) + 3 \times (\% \text{ trienoics}) + 4 \times (\% \text{ tetraenoics}) + 5 \times (\% \text{ pentaenoics}) + 6 \times (\% \text{ hexaenoics})$	Seaweeds [27–29,59,96–98], crops [61,99,100], meat [44,101], and milk [102]
7	EPA + DHA	Sum of eicosapentaenoic acid and docosahexaenoic acid	$\text{C22:6 n-3} + \text{C20:5 n-3}$	Shellfish [34] and fish [34,36,37,40,68,103–106]
8	FLQ	Fish lipid quality/flesh Lipid quality	$100 \times (\text{C22:6 n-3} + \text{C20:5 n-3})/\Sigma\text{FA}$	Fish [65,66,73,107,108]
9	LA/ALA	Linoleic acid / α -linolenic acid ratio	$\text{C18:2 n-6}/\text{C18:3 n-3}$	Lamb [43] and milk [55,109]
10	TFA	Trans fatty acid	ΣTFA	Seaweeds [90], plant oil [32,33,110], fish [35], lamb [45], and milk [78]

NUTRITIONAL QUALITY

Index	Full Name	Calculation Formula
PUFA/SFA	Polyunsaturated fatty acid/saturated fatty acid ratio	$\Sigma\text{PUFA}/\Sigma\text{SFA}$
IA	Index of atherogenicity	$[\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}]/\Sigma\text{UFA}$
IT	Index of thrombogenicity	$(\text{C14:0} + \text{C16:0} + \text{C18:0})/[(0.5 \times \Sigma\text{MUFA}) + (0.5 \times \Sigma\text{n-6 PUFA}) + (3 \times \Sigma\text{n-3 PUFA}) + (\text{n-3/n-6})]$
HH	Hypocholesterolemic /hypercholesterolemic ratio	$(\text{cis-C18:1} + \Sigma\text{PUFA})/(\text{C12:0} + \text{C14:0} + \text{C16:0})$
HPI	Health-promoting index	$\Sigma\text{UFA}/[\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}]$
UI	Unsaturation index	$1 \times (\% \text{ monoenoics}) + 2 \times (\% \text{ dienoics}) + 3 \times (\% \text{ trienoics}) + 4 \times (\% \text{ tetraenoics}) + 5 \times (\% \text{ pentaenoics}) + 6 \times (\% \text{ hexaenoics})$
EPA + DHA	Sum of eicosapentaenoic acid and docosaheptaenoic acid	$\text{C22:6 n-3} + \text{C20:5 n-3}$
LA/ALA	Linoleic acid / α -linolenic acid ratio	$\text{C18:2 n-6}/\text{C18:3 n-3}$
TFA	<i>Trans</i> fatty acid	ΣTFA

Based on MIR performances, 7 from 9 could be predicted by MIR on a routine basis !

Butter spreadability

Spreadability index = C16:0 / C18:1

Increasing the proportion of fresh grass in the diet

→ increase in unsaturated fatty acids percentages at the expense of saturated fatty acids

→ decrease of melting temperature and firmness in mouth

Couvreur et al., 2006, J. Dairy Sci., [https://doi.org/10.3168/jds.S0022-0302\(06\)72263-9](https://doi.org/10.3168/jds.S0022-0302(06)72263-9)

Aspect	Importance for Butter	Main Indicators / Parameters	Link with Milk Fatty Acids
Texture	Determines spreadability and firmness	Hardness, melting point	Higher unsaturated FA → softer butter
Flavor	Influences sensory quality and aroma	Volatile compounds, lipolysis	Short-chain FA (C4:0–C10:0) contribute to flavor

*Milk fatty acids are no longer considered only as milk components; they are now viewed as biomarkers of **nutrition, metabolism, sustainability, and technological quality**.*

CONCLUSIONS



MIR Fatty acids equations

- At least 11 teams:
 - 8 from the scientific community
 - 3 from spectrometer providers
- Different equations
- No possibility to compare performances
- Spectral variability/ reproducibility
- Fatty acids reference measurements



Need for
standardization
to ensure quality
predictions





MeyGen

How much the spectral variability is representative of the cow population?

→ WP3

References hard to obtain leading a limited dataset

Spectral variability

Spectral representativity

How to standardize the signal ?

→ WP3

Different spectral resolutions and signal instability

Equation performances

Every team has its own validation set

Validation performances are not comparable

→ WP2

Different teams develop equations using different references (FA extraction, GC ...)

Reference used to create the equation

Sources of variation

Calibration dataset used

Spectral standardisation

How much are they comparable ?

→ WP1

How much the spectral variability is representative of the cow population?

→ WP3

References hard to obtain leading a limited dataset

Spectral variability

Spectral representativity

How to standardize the signal ?

→ WP3

Different spectral resolutions and signal instability

Equation performances

Every team has its own validation set

Validation performances are not comparable

→ WP2

Different teams develop equations using different references (FA extraction, GC ...)

Reference used to create the equation

Sources of variation

Calibration dataset used

Spectral standardisation

How much are they comparable ?

→ WP1



WP 1 : Reference

Josée Bordeleau
Mazen Bahadi

On behalf of the WP1 participants





As part of the international **ExtraMIR FA project**, ICAR and IDF are collaborating to build a global database of milk fatty acid profiles measured by mid-infrared (MIR) spectroscopy. This database aims to support research, innovation, instrument calibration, and the development of services for producers.

- Josée Bordeleau
- 50 Participants

WP1: Reference



- Action 1.1: Review about the reference methods for quantifying fatty acids in milk
- Action 1.2: Develop procedures to standardize reference fatty acid contents
- Action 1.3: Field Trials
- ~~Action 1.4: Define what data should be collected and covered by the agreement~~
- ~~Action 1.5: Revise a data agreement~~
- Action 1.6: Contact the involved teams for data exchange
- Action 1.7: Set up a data exchange tool on ICAR server [VPN]
- Action 1.8: Creation of a database with reference data coming from different reference measurements

Factors that can influence the results: ensure continuity and comparability of results

GC Reference Method :

- GC Analysis conditions: Column used, length, temperature, etc
- Quality of peaks separation, baseline quality, integration
- Sample prep: Fat extracted from milk or direct milk sample analysis
- Method used to determine total fat and recovery
- Choice of Unit to report data
- List of FA to consider (15-20 FA, Others?)
- Which FA belongs to which group (Mix, Preformed and Denovo)
- Type of internal standards used to provide quantification

Critical!

Choice of GC method used: 2 ISO/IDF methods currently available



Review of the
current reference
methods: Two
ISO\IDF reference
methods exist

ISO 15885 | IDF 184:2002

Milk fat — Determination of the fatty acid composition by gas-liquid chromatography



Specifies a method for the determination of the fatty acid **composition of milk fat and fat obtained from dairy products**. Older method, validated on fat extracts from milk and dairy products, applicable only to milk fat obtained by extraction, rather than to whole milk samples.

- Determination of milk fat fatty acid composition
- Applicable to extracted fat from milk and dairy products
- Analysis performed by gas chromatography (GC)
- Historical and widely used laboratory reference method
- Not designed for direct liquid milk analysis or absolute quantification

Review of the
current reference
methods: Two
ISO\IDF reference
methods exist.

ISO 16958 | IDF 231:2015

***Milk, milk products, infant formulas and adult nutritionals —
Determination of fatty acid composition — Capillary gas
chromatographic method***



This method was **specifically developed for milk and dairy products**, validated on UHT whole milk, milk powder and anhydrous milk fat (18 participating laboratories), and is internationally recognized (Codex, CEN, WHO, China).

- Equivalent to AOAC Official Method 2012.13GC
- Method specifically developed for milk and dairy products
- Validated on whole milk, milk powder, and anhydrous milk fat
- International collaborative validation (18 laboratories)
- Internationally recognized by Codex, IDF, WHO, and China

Portrait of the Situation: GC and IR Performance

**PT tests: the
best available
option**

2 Proficiency Tests (PT) for Fatty Acids (FA)

Reference values – Gas chromatography

Infrared prediction of Fatty Acids concentration

2 Rounds

March 2024

GC: 7 labs and 2 methods

IR: 16 labs

September 2024

GC: 9 labs and 3 methods

IR: 15 labs

PT tests Summary of Findings

**We need
more
valuable
data to do
statistical
analysis!**

GC PT Tests:

- Using same method obtain similar results.
- Both methods gave different results on the same sample set
- Difference around 12% in reported total FA
- Some FA concentrations differ (Long Chain + unsaturated)
- Higher difference with High Fat samples
- More robust statistical analysis is needed.

InfraRed PT Tests:

- Fatty acids results vary greatly between participating labs.
- This distribution explained by multiple factors:
 - Type of instrument (Prediction model)
 - Calibration kit, frequency of calibration
 - Reporting units
- Harmonisation between laboratories is crucial if results are to be compared.





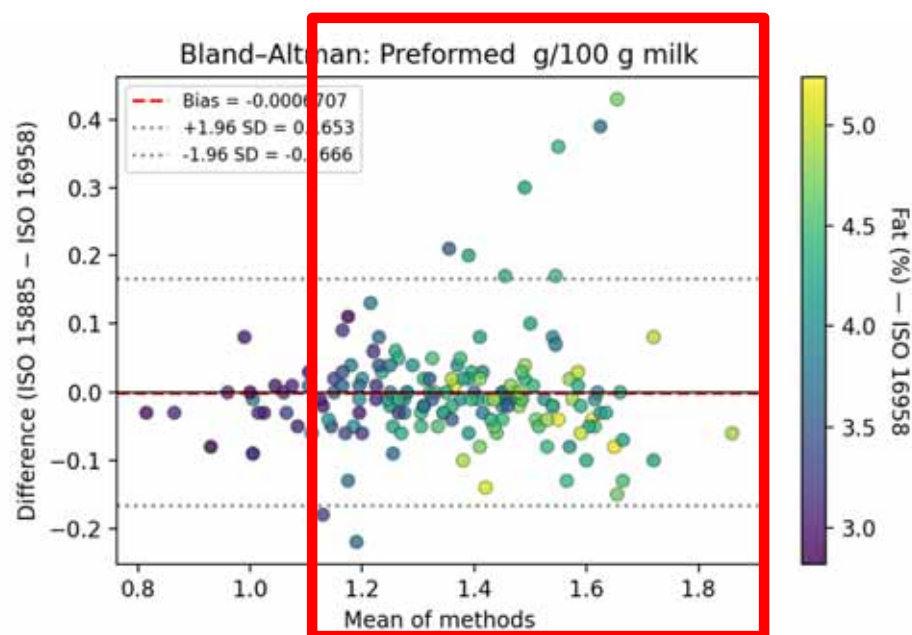
Thank You

- We reached out to the ExtraMIR community to share data that they have
- Same sample, raw or homogenized milk, analyzed by methods ISO 16958 / IDF 231 & ISO15885 / IDF 184
- Datasets were received from:
 - **Malia Caputo – CDCB (USA)**
 - **Philippe Trossat – Actalia (France)**
 - **Kevin van Cleef – Friesland Campina (The Netherlands)**
 - **Octave Christophe – CRA-W (Belgium)**
- Results from two selected datasets will be presented, raw vs homogenized milk
- Results from **all datasets** were **consistent** in their findings

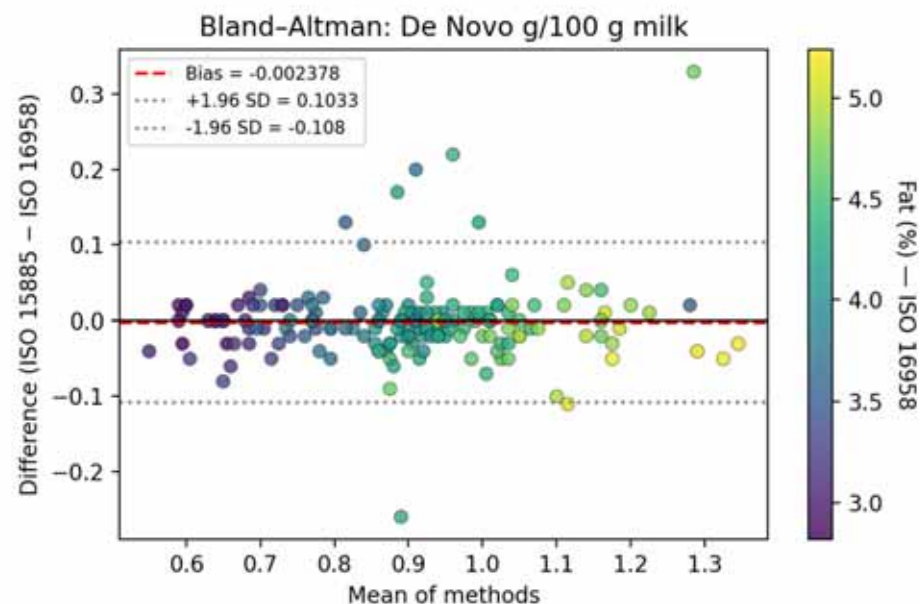


Raw Milk

Preformed FA



De Novo FA



- **Raw milk**, 14 batches, 12 samples per batch, each sample analyzed by ISO15885 and ISO 16958
- At **lower preformed** FA (~0.9–1.1 g/100 g milk), **differences** mostly within **±5–10%**
- At **higher values** (~1.5–1.8 g/100 g milk), **differences** within **±15–25%**
- More positive percent differences, **ISO 15885** tends to report **higher** values than **ISO 16958** at **higher concentrations**



Statistical Analysis

For each **fatty acid trait**, a **linear mixed-effects** model was fitted:

$$Y \sim \textit{method} + \textit{Fat}_c + \textit{method} * \textit{Fat}_c + \textit{batch} + (1|\textit{Sample ID})$$

- where ***method*** (ISO 15885 vs ISO 16958), **centered fat concentration** ($\textit{Fat}_c$), their **interaction**, and analytical **batch** were included as **fixed effects**, while **sample identity** was included as a **random intercept** to account for paired measurements of the same milk sample analyzed by both methods.
- Kenward–Roger correction of degrees of freedom

Results



	Method	Fat	Fat * Method	Batch
		P values		
Total FA	0.0011	<.0001	0.0791	<.0001
De Novo g/100 g TFA	0.001	<.0001	0.6176	0.9349
Mixed g/100 g TFA	<.0001	<.0001	0.212	0.2435
Preformed g/100 g TFA	0.0002	<.0001	0.1974	0.4666

- How are **total fatty acids** determined for each method?
- Are the **same units** measuring the **same quantities** for both methods?

Results

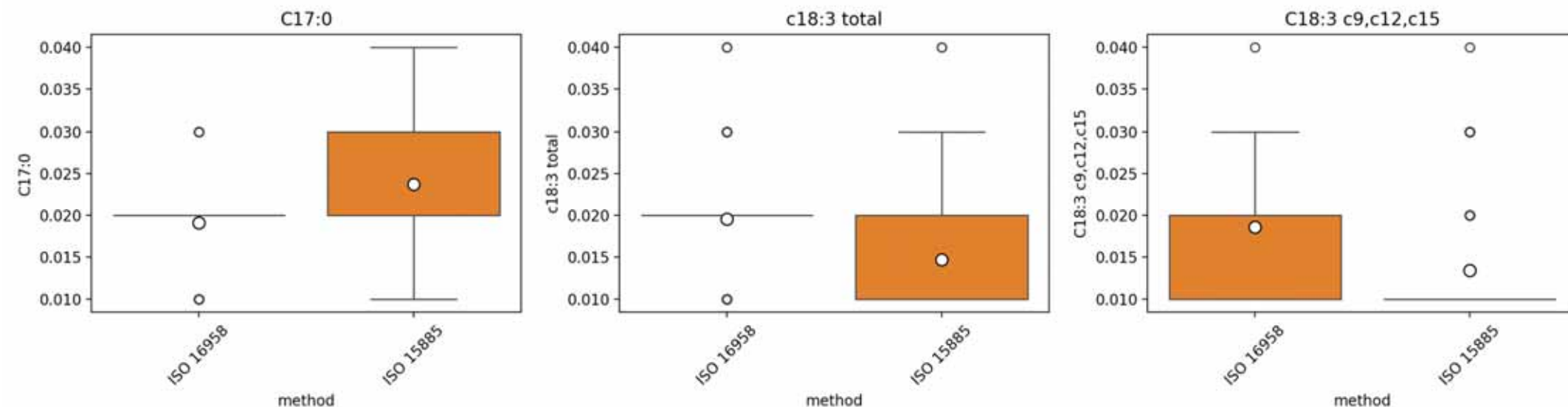


	Method	Fat	Fat * Method	Batch
		P values		
C4:0 g/100 g milk	<.0001	<.0001	0.8284	<.0001
C6:0 g/100 g milk*	0.0053	<.0001	0.3361	0.0015
C8:0 g/100 g milk*	<.0001	<.0001	0.1979	0.2447
C10:0 g/100 g milk	<.0001	<.0001	0.5399	0.781
C12:0 g/100 g milk	0.0122	<.0001	0.3979	0.9878
C14:0 g/100 g milk	0.594	<.0001	0.5011	0.3589
C14:1 c9 g/100 g milk*	<.0001	<.0001	0.9906	0.0063
C15:0 g/100 g milk*	0.0028	<.0001	0.6057	0.0532
C16:0 g/100 g milk	0.1427	<.0001	0.1057	0.0003
C16:1 c9 g/100 g milk*	<.0001	<.0001	0.0311	<.0001
C18:0 g/100 g milk	0.0353	<.0001	0.2439	0.0069
C18:1 c9 g/100 g milk	0.0038	<.0001	0.4507	0.0015
C18:2 c9,c12 g/100 g milk	0.0046	0.0176	0.7192	0.4226

* max. concentration < 0.1g/100g milk



Results



- Are we using the same peak(s) for the same fatty acid(s)?
- Are these **peaks well resolved** in both methods?



Homogenized milk products

Relative Differences % (ISO 16958 - ISO 15885)			
Sample ID	De Novo	Mixed	Preformed
Whole milk powder	-0.10	-1.36	-1.24
Evaporated milk	0.18	-1.51	-0.76
Semi-skimmed milk	1.70	-2.21	-2.00
Infant formula 1	-0.94	-0.05	0.31
Infant formula 2	2.32	-1.70	-0.69

- Limited number of samples
- The first three samples are made from **homogenized milk**, **fat content was not provided**
- Infant formula **does not contain bovine fat milk**, it is usually replaced by vegetable oil
- **No fat globules**
- When **fatty acids** are **released** by the transesterification reaction, the **differences** between the two methods are **minimal**



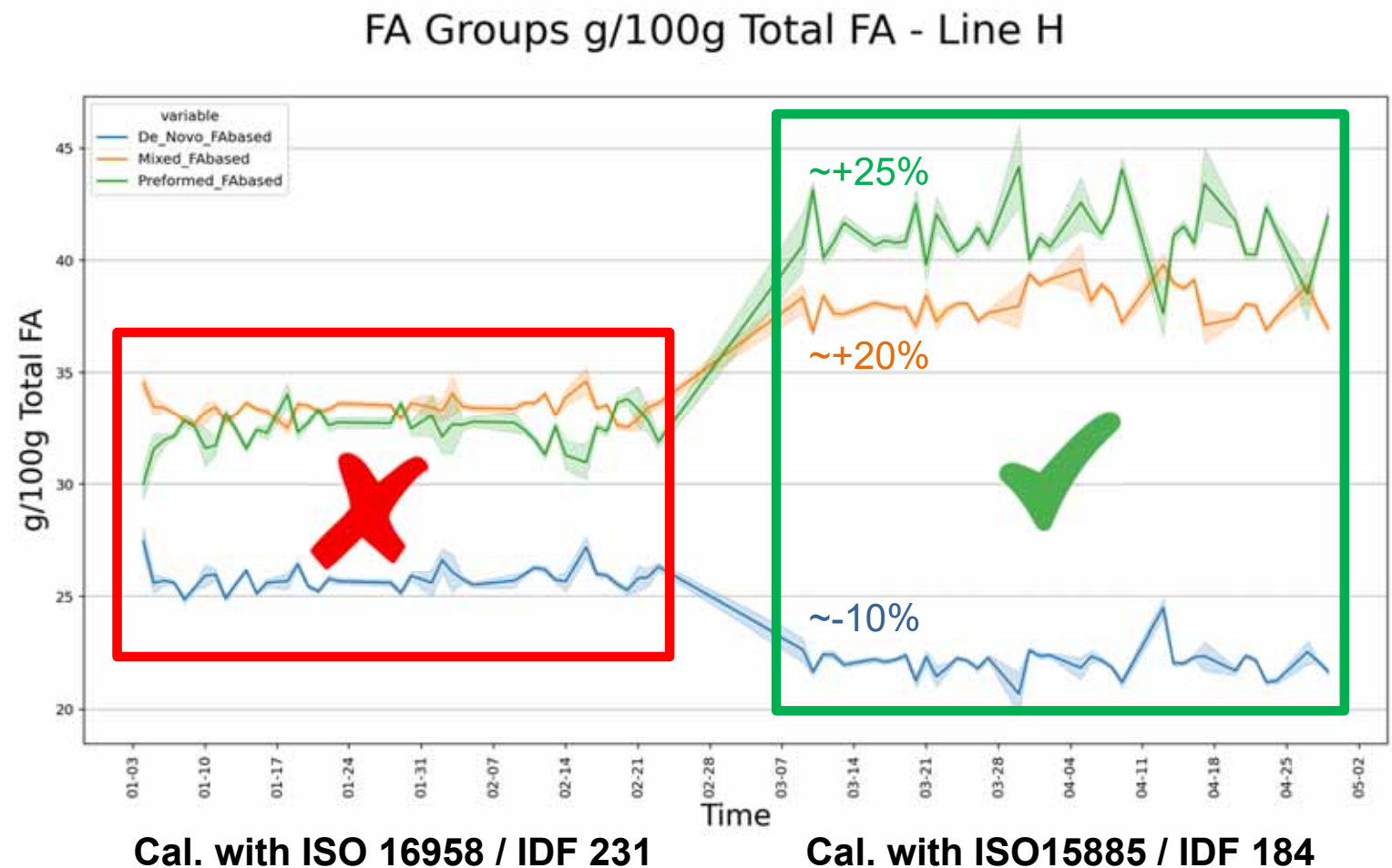
Milk Ultrasonication

- Milk sample with 4.27% fat
- Different combinations of sonication time and amplitude, energy delivered 3,000-20,000 joules
- **Significant improvements** were observed for **recovered fatty acids**:
 - **Total FA: >+5% (p-value = 0.004)**
Total FA recovered from fat increased from ~86% to ~92%
 - De Novo +1.3% (p-value = 0.007)
 - Mixed: +2.2% (p-value < 0.001)
 - Preformed: +2% (p-value = 0.007)

Milk FA Profile in Ind. Cows Samples Jan-Apr 2026



- **IR results** for De Novo, Mixed and Preformed FA
- The only difference is that the analyzer was calibrated with a calibration kit analyzed by ISO15885 / IDF 184 on March 4th
- ISO15885 / IDF 184 results align better with the **current scientific knowledge** and **nutrition data** we have





Factual Observations

- When applied to raw milk, ISO 16958 | IDF 231 and ISO 15885 | IDF 184 may produce **different fatty acid results in certain cases**, particularly at **higher milk fat contents**.
- Differences are **less evident** in **homogenized** or **heat-treated** milk products.
- In raw milk, **access of reagents to milk fat globules during transesterification may be incomplete**, potentially leading to lower fatty acid recovery.
- Additional sample disruption (e.g. **sonication** or modified sample preparation) was shown to **increase** fatty acid **recovery** in raw milk.
- When **overall means** are used to compare datasets, **apparent agreement** between methods may conceal **fat-dependent effects**.



Next Steps

- IDF created an action team to improve the applicability of ISO 16958 / IDF 231 to raw milk
- Suggested points to be considered
 - Are **all fatty acids** being released after the **transesterification reaction** from **fat globules** in raw milk?
 - What is meant by “**total fatty acids**” for each method and how are they determined?
 - Are **peaks** of individual fatty acids **comparable** in both methods?
 - Are results similar when expressed in **different units** (g/100g milk vs g/100g TFA) for both methods?
 - The method to **quantify fat** and how **TFA recovery** is determined.

How much the spectral variability is representative of the cow population?
→ WP3

→ WP3

References hard to obtain leading a limited dataset

Spectral variability

Spectral representativity

Sources of variation

Equation performances

Every team has its own validation set

Validation performances are not comparable
→ WP2

Reference used to create the equation

Different teams develop equations using different references (FA extraction, GC ...)

How to standardize the signal ?

→ WP3

Different spectral resolutions and signal instability

Calibration dataset used

Spectral standardisation

How much are they comparable ?

→ WP1



WP2 : Modelling

- Steve Holroyd
- 43 Participants

Action 2.1: Realize an inventory of existing equations to predict fatty acids in milk.

Action 2.2: Contact the involved teams for data exchange to create the international validation set

Action 2.3: Write a data agreement to create the validation set.

Action 2.4: Set up a data exchange tool on ICAR server.

Action 2.5: Create a database with spectral and reference fatty acid data on ICAR server

~~**Action 2.6:** Develop equations with all available data by testing different methodologies~~

Action 2.7: Create the international validation set with data available in Action 2.5.

Action 2.8: Create a web interface [Rshiny] to validate the equations with the validation developed in Action 2.7.



Action 2.7: Create the international validation set

- A first dataset was created to run the draft of the Validation Rshiny tool
- This validation dataset must be improved using data that will be sent by the partners who signed the agreement (Action 2.2)



WORK IN PROGRESS

This WP was on a pause mode awaiting the results of the WP1 fatty acids PT to construct the international validation set.

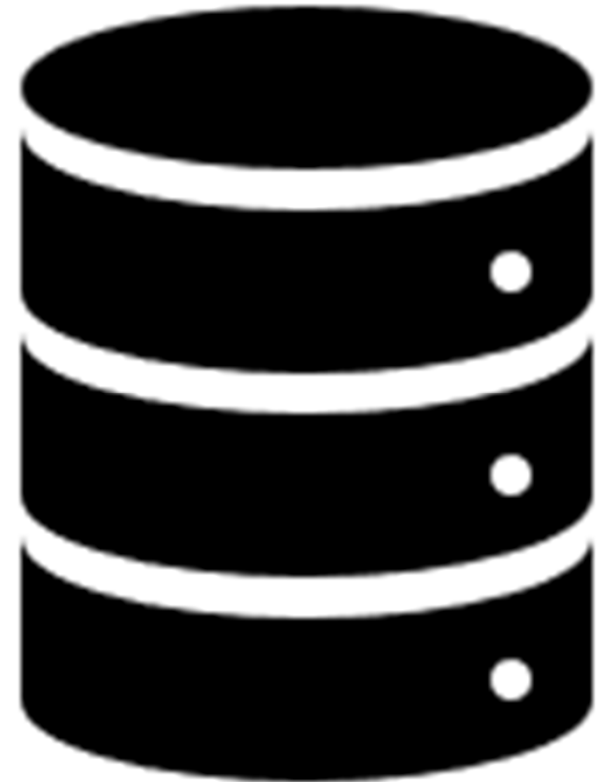
WP1 concludes that the two GC method (ISO16958/IDF231 and ISO15885/IDF184) provides different results.



What are the consequences on the MIR-based fatty acid predictions?

Data

- 194 bovine individual and bulk milk samples
- Analyzed by Octave Christophe at the CRA-W (Gembloux Belgium) using Method2 (ISO16958/IDF231)
- MIR analysis performed by Comité du Lait (Battice, Belgium) on a Foss MilkoScan spectrometer
- Spectra were standardized using the method of Grelet et al. (2018)
- Fatty acids were predicted on those spectra using PLS equations developed from milk samples analyzed by Method 1 (ISO15885/IDF194) → PLS1
- Only FA with a calibration PLS1 performance > 75% were retained (19 traits)

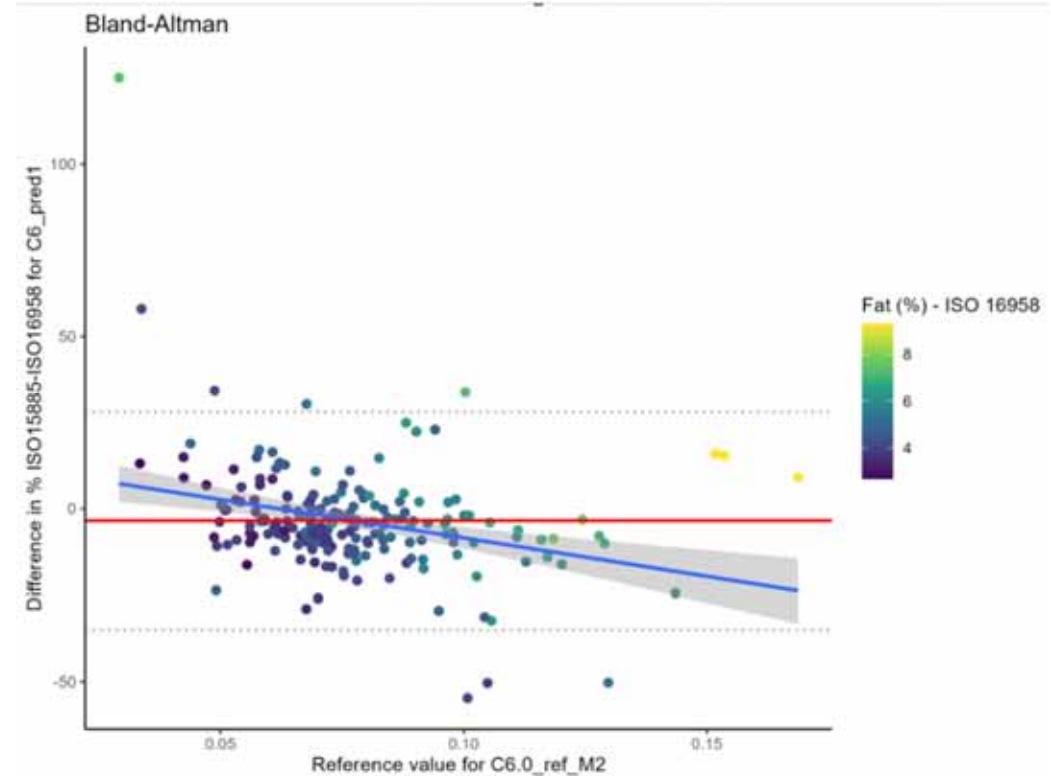
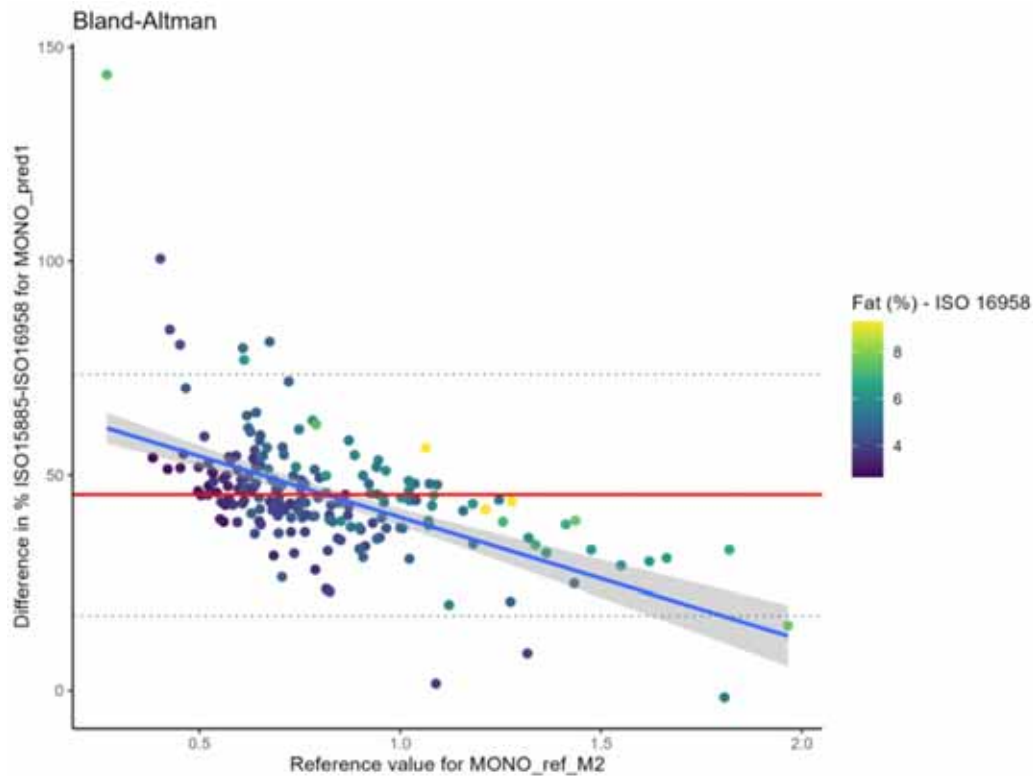


FA difference : Method2 – Method1

- P-value of Paired t-test higher lower than 5% for all studied traits → significance difference between reference Method 2 and PLS1 prediction (Method 1)
- Negative difference except for C6:0, LCFA, C4:0 and total C18:1 trans

Trait	N	Mean	SD	mean difference
SAT	194	2.477	0.645	-0.78863
C14:0	194	0.396	0.101	-0.14285
MCFA	194	2.173	0.522	-0.34202
C16:0	194	1.192	0.335	-0.42777
C12:0	194	0.114	0.036	-0.02109
INSAT	194	0.897	0.304	-0.51129
MONO	194	0.811	0.283	-0.45971
C10:0	194	0.094	0.029	-0.01129
C8:0	194	0.042	0.013	-0.00374
SCFA	194	0.353	0.098	-0.01292
totC18:1	194	0.835	0.295	-0.26299
C18:1cis9	194	0.660	0.245	-0.27676
totC18:1cis	194	0.724	0.264	-0.27373
C6:0	194	0.077	0.022	0.00266
LCFA	194	2.793	1.011	1.01131
POLY	194	0.086	0.025	-0.04575
C18:0	194	0.346	0.142	-0.12357
C4:0	194	0.141	0.042	0.01320
totC18:1trans	194	0.111	0.044	0.00903
DeNovo	194	0.911	0.233	-0.18897
Mixed	194	1.203	0.335	-0.51082
Preformed	194	1.342	0.456	-0.41911

Bias



Bias ranged from -57% to 36%.

Percentage biased with an absolute value below 10% : C4:0, C6:0, C8:0, total C18:1 trans and SCFA.

Largest bias in absolute value : UFA, PUFA and MUFA

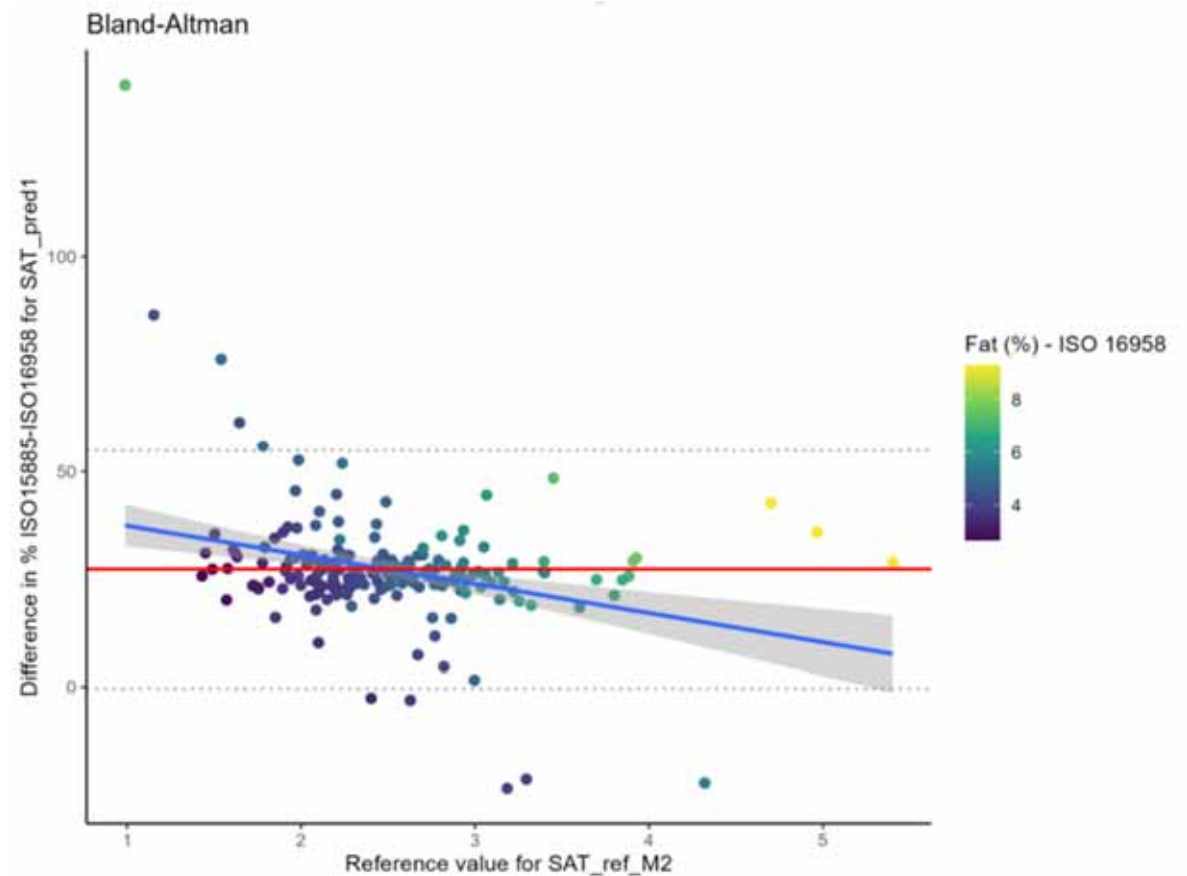
RMSE : Method2 – Method1

- Observed RMSE (PLS1 prediction vs. reference Method2) (*erreur_reg*)
- Model PLS1 RMSE (*erreur_model*)
- Differences expressed in % (*%diff_erreur*)
- For all fatty acids, observed RMSE was greater than expected, except for total C18:1 trans.
- Strong difference for SAT known to be well predicted by MIR. → GC differences

Trait	N	erreur_reg	erreur_model	%diff_erreur
SAT	194	0.4789	0.0749	16.31
C14:0	194	0.0932	0.0334	15.07
MCFA	194	0.4198	0.1181	13.88
C16:0	194	0.2485	0.1003	12.44
C12:0	194	0.0282	0.0141	12.40
INSAT	194	0.1795	0.0732	11.85
MONO	194	0.1584	0.0662	11.37
C10:0	194	0.0224	0.0121	10.98
C8:0	194	0.0087	0.0046	9.70
SCFA	194	0.0602	0.0273	9.31
totC18:1	194	0.1361	0.0690	8.03
C18:1cis9	194	0.1209	0.0681	7.99
totC18:1cis	194	0.1249	0.0692	7.70
C6:0	194	0.0126	0.0067	7.68
LCFA	194	0.3321	0.1200	7.59
POLY	194	0.0285	0.0238	5.48
C18:0	194	0.0786	0.0638	4.29
C4:0	194	0.0155	0.0096	4.17
totC18:1trans	194	0.0226	0.0304	-6.99
DeNovo	194	0.1826		
Mixed	194	0.2635		
Preformed	194	0.2326		

Effect of fat content

- SAT is well predicted by MIR
- Impact of fat content is visible



Effect of fat content

- Effect of fat content on the difference between reference measured using Method2 and prediction obtained from Method1
- All significant except total C18:1 trans
- Strongest impact on UFA (INSAT), C14:0, PUFA (POLY), and C12:0

Trait	Estimate	Estimate_SE	p-valeur	R2
INSAT	0.10791	0.00865	1.57E-26	0.4475
C14:0	0.05336	0.00463	1.13E-23	0.4086
POLY	0.01609	0.00140	1.18E-23	0.4083
C12:0	0.01656	0.00146	4.08E-23	0.4007
MONO	0.08865	0.00794	1.21E-22	0.3939
C10:0	0.01275	0.00116	5.06E-22	0.3849
SAT	0.26097	0.02431	2.32E-21	0.3751
Mixed	0.14019	0.01341	1.55E-20	0.3627
MCFA	0.22294	0.02133	1.56E-20	0.3627
De Novo	0.09225	0.00965	6.01E-18	0.3223
C16:0	0.12107	0.01316	6.06E-17	0.3060
C18:1 cis-9	0.05350	0.00664	8.11E-14	0.2527
C8:0	0.00376	0.00048	4.33E-13	0.2397
Preformed	0.09472	0.01350	3.86E-11	0.2040
tot C18:1cis	0.04905	0.00714	8.77E-11	0.1973
totC18:1	0.04961	0.00797	2.95E-09	0.1680
SCFA	0.01736	0.00365	3.87E-06	0.1054
C18:0	0.02288	0.00533	2.76E-05	0.0877
LCFA	-0.16385	0.04515	0.00036468	0.0642
C6:0	0.00244	0.00082	0.00317807	0.0444
C4:0	-0.00411	0.00142	0.00423585	0.0418
tot C18:1 trans	-0.00081	0.00175	0.64253717	0.0011

Conclusions & Limitations

- Not appropriate to provide predictions estimated from equations based on Method 1 to clients relying on Method 2 without applying a correction
- Limitations :
 - Both Methods are not available for the 194 samples.
 - Comparison of Method 2 reference with Method 1 predictions → the prediction error is also included in the observed differences
- But, conclusions confirm the WP1 findings on GC comparison for the fat content and the over-estimation of Method1 compared to Method2 for the majority of FA

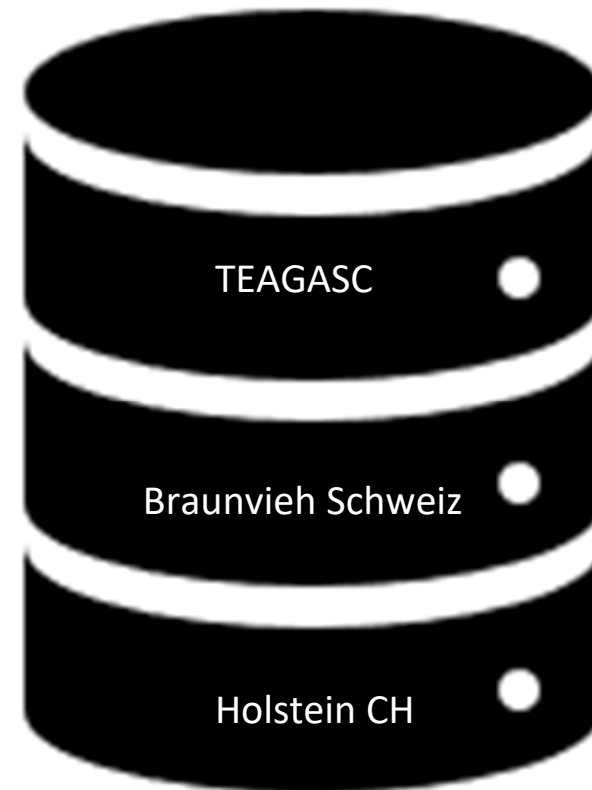
Perspectives

- If Method2 is confirmed to be better than Method 1, two possible corrections:
 - **Short term** : A posteriori correction based on a calibration kit to correct the values after predictions using an equation developed with Method1 samples
 - **Longer term** : A new development of equations by including in the training samples analyzed using the Method2
- In the WP2, information about the GC analysis will be asked to create the international validation dataset in order to consider separately the data.



International
validation set

We need you ...



Action 2.7: Create the international validation set

- A first dataset was created to run the draft of the Validation Rshiny tool
- This validation dataset must be improved using data that will be sent by the partners who signed the agreement (Action 2.2)
- Need to respect the format of the database (Action 2.5)
- Take into account the outputs of the WG1 about the fatty acids or groups that can be compared even if different reference methods were used



WORK IN PROGRESS

Action 2.8: Create a web interface [Rshiny] to validate FA equations

A first version is available and ready to be tried by ExtraMIR members



GOAL:

The validation tool will allow a users to compare their calibration equations/predictions for fatty acids against a representative calibration and assess how similar /different the predictions are.

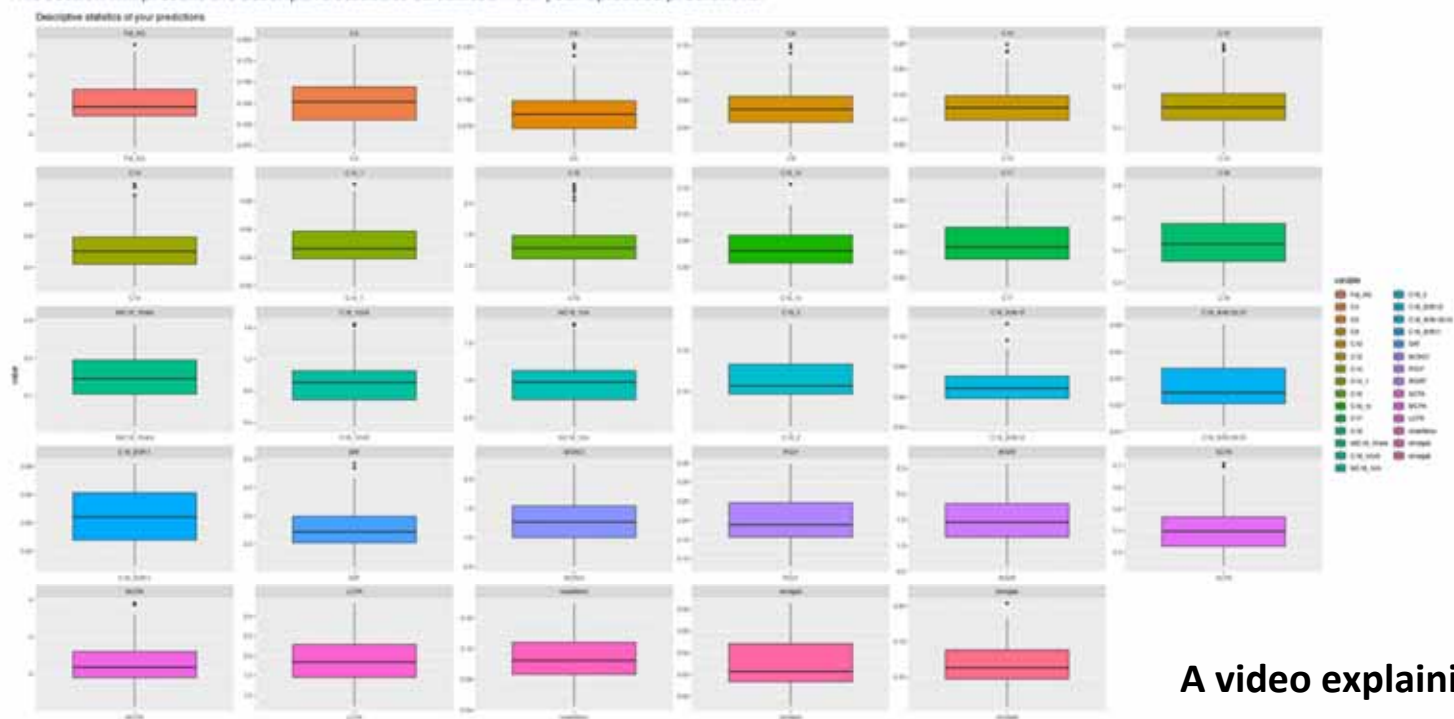
An **identification module** was added with ID and password provided by ExtraMIR coordinators.



ExtraMIR - Validation tool

User Identification Download Validation Spectral Dataset Upload your Obtained Prediction Dataset Statistics of your Uploaded Predictions Validation performances of your equations Report Download Data sharing agreement

This section will present the descriptive statistics calculated from your uploaded predictions.



A video explaining how to use the tool will be produced soon.

WP2 – next steps

- If the Method2 is the reference method : Study the possibility of creating mixing training set to solve the issue of Method2 difference.
- Create a video to explain how to use the developed Validation tool installed on ICAR server.
- Collect the comments from ExtraMIR WG2 members for the Validation tool to finalize it.
- Contact potential extra partners that can share FA data to complete the validation set.
- Communicate about the tool and the interest of using FA depending on the stakeholders (connexion with WG4).

WP 3: Quality Assurance

QUALITY CONTROL





WP3 : Quality assurance

WP leader : Frédéric Dehareng

Action 3.1: Contact partners with milk spectral data to create the World Representative Spectral Database (WRSD)

Action 3.2: Write a data agreement for WRSD

Action 3.3: Create the WRSD

Action 3.4: Create a web interface (R shiny) to assess the representativeness of a calibration set on WRSD

Action 3.5: Compare methods for spectral standardization

~~**Action 3.6:** Develop an open script for the detection of outlier predictions~~

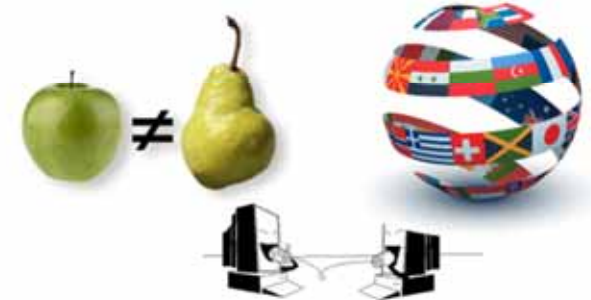


Objective and importance of MIR spectral standardization



Harmonization of spectra across instruments, labs, countries, continents

- Sharing of model
- Sharing of data (costly reference)



Harmonization of spectra across time

- Useability of models in time
- Stability of predictions



Harmonization of historical database

- Use of new models on historical spectra
- Power of big data
- Genetic evaluation



Existing methods

- Bentley (integrated in the instrument, Bentley)
- Foss (analysis of common samples, Foss)
- CRA-W/EMR PDS (analysis of common samples, All brands)
- Lactanet RPS (use of historical datasets, Foss)
- VIT (use of historical datasets, Foss) + others PDS std in N-Z, in China...

ExtraMIR 3.5

- Compare the existing methodologies
- Communicate on results



Action 3.5: Compare methods for spectral standardization



- Inventory of existing methods
- Consensus on the methodology/protocol to be applied
- Comparison of 4 different methods
- Results presentation and discussion
- Consensus on how to present the results
- Submission of the publication for peer review
- Certification of standardization methods

Since Anand 2025 ...

9 sept. 2025

Open online meeting to present once again the results

- Reminder of WP3 objectives and status update following Anand's meeting
- Specific point on standardization: results and conclusion regarding the comparison of standardization methods4Next steps : publication of results? Discussion on the opportunity to propose a certification for the standardization methods

5 nov. 2025

ExtraMIR WP leader meeting

- Reviewing project progress and preparing for upcoming IDF standing committee meetings

17 oct. 2025

ICAR MA SC meeting

- ExtraMIR WP3 – working group organization



Since Anand 2025 ...



19 nov. 2025

ExtraMIR 3.5 group meeting :

- First group to discuss on certification

28 nov. 2025

IDF SCLSQA – S20- IDF ICAR ExtraMIR

- Presentation of the results, conclusions and next steps (publication + certification)

20 nov. 2025

ExtraMIR 3.5 group meeting:

- Communication meeting (strategy, methodology)

14 avr. 2026

ExtraMIR 3.5 group meeting :
communication meeting (decision on
publication):

Publication decisions (14/04/2026)



Differences in spectral standardization may **limit model transferability** and require further investigation



The reproducibility of **laboratory fat predictions** (slope bias correction) is retained as the **reference threshold**



VIT fat predictions still need to be validated before deciding on their inclusion or on model application



The comparison of methods relies on **one manufacturer as a baseline**, representing a **preliminary quality assurance step**

Publication decisions (14/04/2026)



Analyses must account for the **imbalance in the experimental design** and the unequal number of instruments



To solve the **problem of anonymity of methods** :

Methods will be described by referring to previously published studies, and the ExtraMIR results are contextualized through citation of these references (e.g., Bonfatti, 2017; Grelet, 2017; Mensching, 2025).



The scientific publication will be **developed collaboratively**, starting with a first draft followed by a collective selection of target journals.

Scientific
publication

Current status



The ExtraMIR 3.5 group is composed of involved standardization stakeholders, including representatives from IDF and ICAR.



All results and conclusions have been reviewed by the 3.5 group, and consensus has been reached regarding their presentation.



A presentation is scheduled for **Thursday** during the ICAR Congress (C. Grelet)



An initial draft of the manuscript has been prepared by M. Frizzarin in collaboration with C. Grelet.

Points for discussion

- Key challenge: **ensuring fairness across manufacturers** in the context of full methodological transparency, particularly given the absence of one manufacturer in the proficiency test (PT).
- Maintaining **publication peer-reviewed journal** remains essential to ensure scientific rigor.
- **Consensus process for publication of results:**
 - Should this involved ExtraMIR 3.5 (experts and stakeholders including IDF and ICAR representative)?
 - Should additional parties be included?
 - The requirement for pre-submission approval could further complicate an already highly intricate process. It may also potentially slow down the workflow due to additional validation constraints.



Potential additional points



Vulgarization paper ? Who ?



A second spectral ring test could be considered, however, questions regarding feasibility remain (rationale, participation, cost, motivation,...)?

Next steps

- **Certification**

- **Ongoing proposal**
- Prepared by C. Grelet following group conclusions
- Rely on criteria already published or required in standards and guidelines (e.g., ISO/IDF standards, ICAR guidelines).
- Further discussed within the group to assess whether a consensus can be reached.



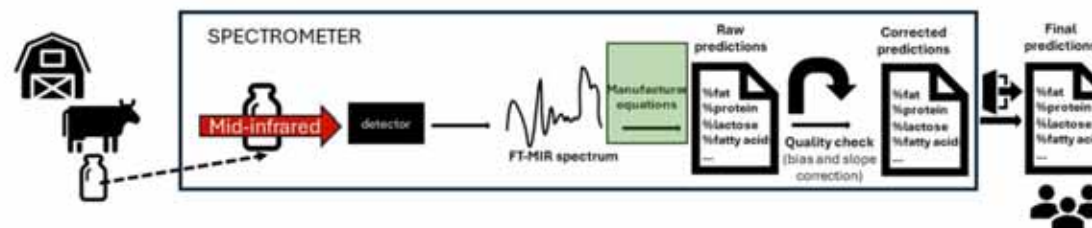
WP 4: Communication

Michael Whittaker



Next steps for WP4

- During the nearly 3 years, we have gained a lot of new results (WRSD, GC comparison, spectral standardisation ...)
- Need to communicate the results to all stakeholders
 - Scientific publications
 - Fact sheets
 - Videos
 - Certification ...



ExtraMIR ICAR/IDF Factsheet The role of fatty acid analysis in enhancing dairy management

This factsheet explores the key applications of fatty acid analysis in dairy cattle management. It details how fatty acid data can enhance herd health and productivity, examines the role of FT-MIR spectroscopy in fatty acid analysis, and showcases examples of current global fatty acid management tools.

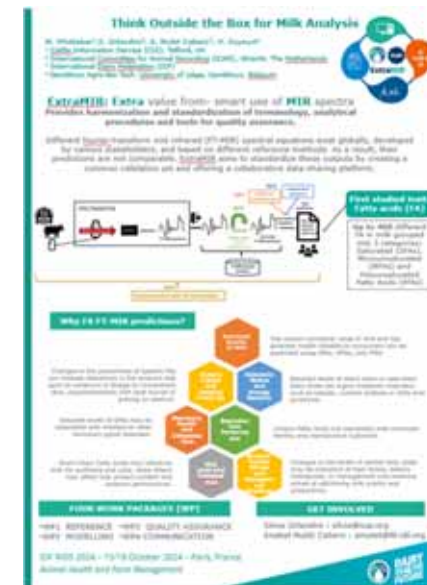
Fatty Acids in Bovine Milk

Bovine milk contains a diverse range of fatty acids (FAs), with up to 400 different types identified (Jensen, 1973). Some of the main FAs found in bovine milk are as follows:

- **Saturated Fatty Acids (SFAs):** These are the most prevalent FAs in milk. Common SFAs in bovine milk include:
 - Palmitic acid (C16:0): This is the most abundant SFA in milk.
 - Stearic acid (C18:0)
 - Myristic acid (C14:0)
- **Monounsaturated Fatty Acids (MUFAs):** These are present in smaller amounts compared to SFAs. The most common MUFA is oleic acid (C18:1).
- **Polysaturated Fatty Acids (PUFAs):** Bovine milk contains small amounts of PUFAs. Notable PUFAs include:
 - Linoleic acid (C18:2), an omega-6 fatty acid.
 - Alpha-linolenic acid (C18:3), an omega-3 fatty acid.
- **Conjugated Linoleic Acid (CLA):** This is a type of polysaturated FA found in bovine milk, known for its potential health benefits (Lock & Bauman, 2004).

Further details on the FA content in milk can be seen in Table 1.

The FA composition of bovine milk varies depending on factors such as the breed of the cow, its diet, and the stage of lactation (Palmquist et al., 1993). Additionally, milk types (e.g., whole milk, skim milk) may have slightly different FA profiles due to variations in fat content (Moate et al., 2005).



Review of Tools Using Fatty Acids in Milk

Bovine milk fatty acids (FA) play a crucial role in human nutrition, dairy science, and industrial applications. Various tools have been developed for the extraction, characterization, and utilization of FA. This review explores these tools, focusing on analytical techniques, bioinformatics resources, and application-specific methodologies. Recent advancements in green extraction techniques and computational modeling have significantly enhanced FA research.

Introduction
Bovine milk contains a diverse range of fatty acids, including saturated (SFA), monounsaturated (MUFA), and polysaturated fatty acids (PUFA). These fatty acids are critical for human health and have functional properties in food and pharmaceutical industries (Parodi, 2004). Advanced tools are necessary for precise extraction, characterization, and application of FA. This review provides an in-depth evaluation of the tools used in FA research and their effectiveness.

Extraction Techniques
Efficient extraction methods are vital for obtaining high-quality FA. The choice of extraction technique affects yield, purity, and bioactivity.

- **Solvent extraction:** A conventional method using organic solvents like hexane or petroleum ether to extract total lipids. It is widely used but requires long processing times and large solvent volumes (Polch et al., 1997).
- **Supercritical Fluid Extraction (SFE):** Utilizes supercritical CO₂ to extract lipids, offering a solvent-free and eco-friendly alternative. SFE provides better selectivity and preserves the bioactivity of unsaturated fatty acids (Invernizzi et al., 2006).
- **Accelerated Solvent Extraction (ASE):** A high-pressure technique using controlled temperature and pressure to reduce solvent consumption while increasing extraction efficiency (Votter et al., 1996).

Analytical Tools for FA Characterisation
To analyse FA, highly sensitive and precise instruments are employed:

- **Gas Chromatography-Mass Spectrometry (GC-MS):** The most widely used tool for fatty acid profiling, offering high-resolution and sensitivity. Fatty acid methyl esters (FAMES) are commonly analysed using this method (Christie, 1993).
- **High-Performance Liquid Chromatography (HPLC):** Used for separating and quantifying fatty acids in their native state, particularly for triacylglycerols and phospholipids (Zhang et al., 2006).
- **Fourier Transform Infrared Spectroscopy (FTIR):** A rapid and non-destructive method for detecting functional groups in BMPAs, useful for quality control (Vongpravit et al., 2014).

Bioinformatics & Computational Tools
Advancements in computational approaches have revolutionised FA research:

- **Lipidomics Software (LIPID MAPS, LipidSearch):** Enables comprehensive analysis of fatty acid profiles, allowing researchers to study metabolic pathways and lipid functions (Pahy et al., 2009).
- **Machine Learning Models:** Predict FA composition based on dairy cattle diet, genetics, and environmental factors, optimising milk production quality (Gang et al., 2021).



Description of the relevant activity/issue proposed by participants :

WP4: Communication

- Michael Whittaker
- 40 Participants

Action 4.1: Review of management tools using fatty acid FT-MIR predictions

Action 4.2: Write a guideline for fatty acid quantification methods

Action 4.3: Develop a guideline for spectral data standardisation

Action 4.4: Write a document informing about the potential use of fatty acid data for dairy farmers and the industry

Action 4.5: Write a report about the communication of equation performances

Action 4.6: Write a guideline to ensure high quality predictions using FT-MIR spectrometry

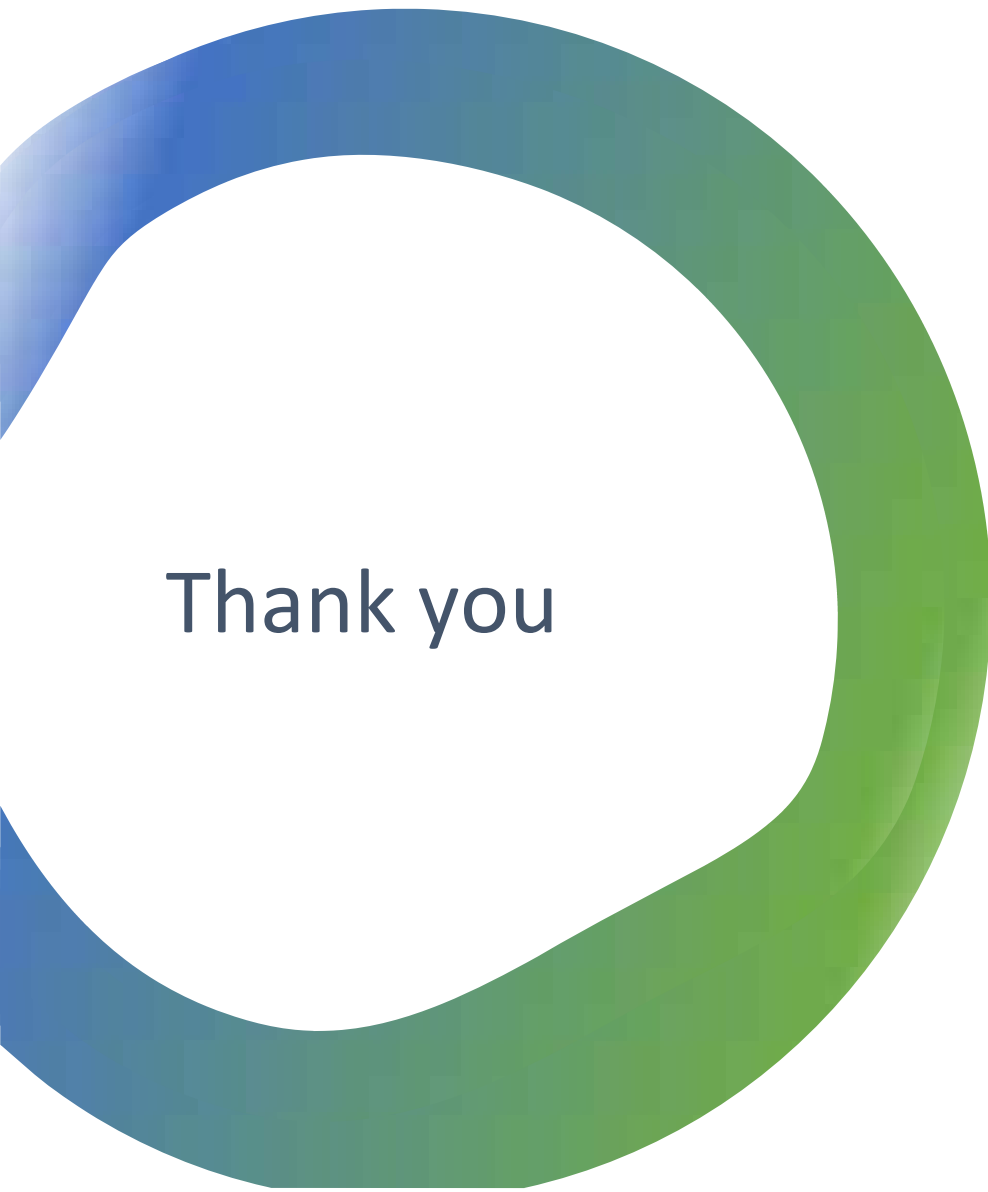
- ✓ 2 videos were produced :
 - ✓ Introduce ExtraMIR project
 - ✓ Explain the use of the WRSD tool
- ✓ Action 4.1. and 4.4. is nearly finished : Fact sheets are written
- ✓ Action 4.2 can start now with the results of the 2 fatty acids proficiency tests done in WG1
- ✓ Action 4.3 : write a vulgarized article (Fact sheet) to explain why it is important to standardize the spectral data + write a document on the output of spectral proficiency test
- ✓ The remaining actions will be related to the output of previous WPs.

How can we improve the communications?

- Collect informations already communicated by partners about :
 - MIR spectrometry ?
 - Fatty acids ?
 - MIR-based fatty acids ?
- And then adapt to create fact sheets, videos ???
- We need help from communicators...
- Ideas ?

Conclusions





Thank you



This year, we have continued to obtain **concrete results.**

Thank you for your contribution !



You want to join the project

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