MILK FATTY ACID ANALYSIS

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ANALYTICS BEYOND MEASURE

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ANALYTICS BEYOND MEASURE

1. INTRODUCTION

EVOLUTION OF UNDERSTANDING OF MILK FATTY ACIDS

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1897	• Question of the origins of milk fat (wholly from diet or synthesised by animal)? (Jordan and Jenter, 1897)
1947	• Leading theory: Short-chain fatty acids arise from degradation of oleic acid (Hilditch, 1947)
1951	• De novo synthesis of short-chain fatty acids proven (Popjak et al., 1951)
1990	• Detailed determination of origin of fatty acids completed (e.g., Palmquist, 2006)
2000s	• Fatty acid profiling (chain length, degree of saturation, major fatty acids) using FTIR technology (FOSS) → Practical applications: Focus on milk processing/dairy product aspects
2010s	• Practical applications: Fatty acid profiling with focus on dairy cow nutrition (Visiolait project)

FATTY ACID CALIBRATIONS AVAILABLE SINCE 2007

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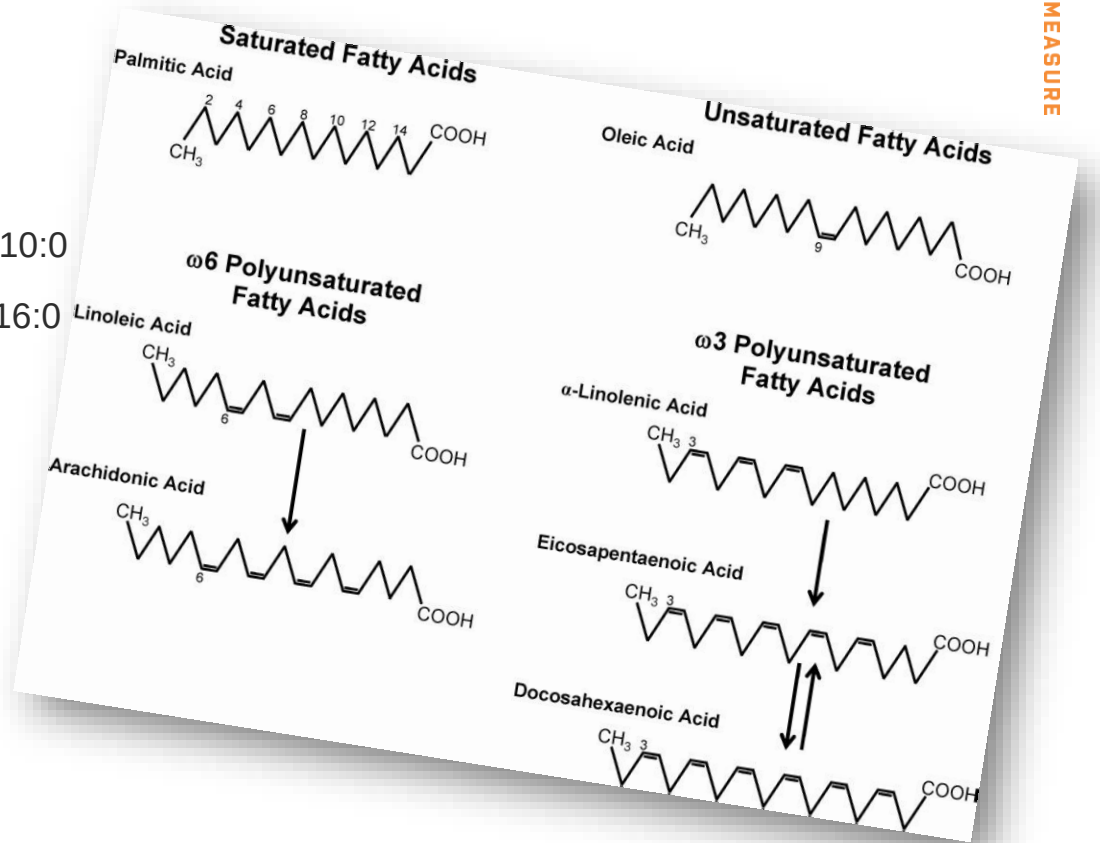
Package I

- Degree of unsaturation
  - Saturated Fatty Acids (SFA)
  - Mono Unsaturated Fatty Acids (MUFA)
  - Poly Unsaturated Fatty Acids (PUFA)
  - Trans FA

Package II

- Chain length
  - Short Chain Fatty Acids (SCFA): $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$
  - Medium Chain Fatty Acids (MCFA): $C_{12:0}$, $C_{14:0}$, $C_{16:0}$
  - Long Chain Fatty Acids (LCFA): $C_{18:0}$, $C_{18:1}$, $C_{18:2}$
- Major fatty acids
  - $C_{14:0}$
  - $C_{16:0}$
  - $C_{18:0}$
  - $C_{18:1}$



FREE FATTY ACIDS VS FATTY ACIDS

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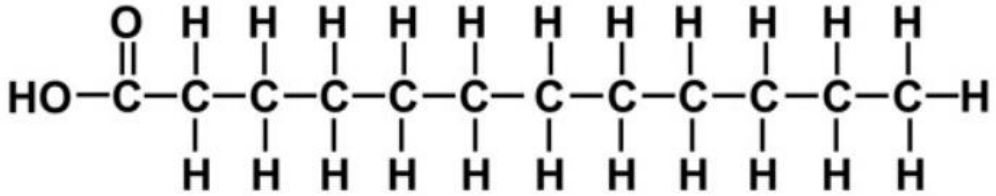
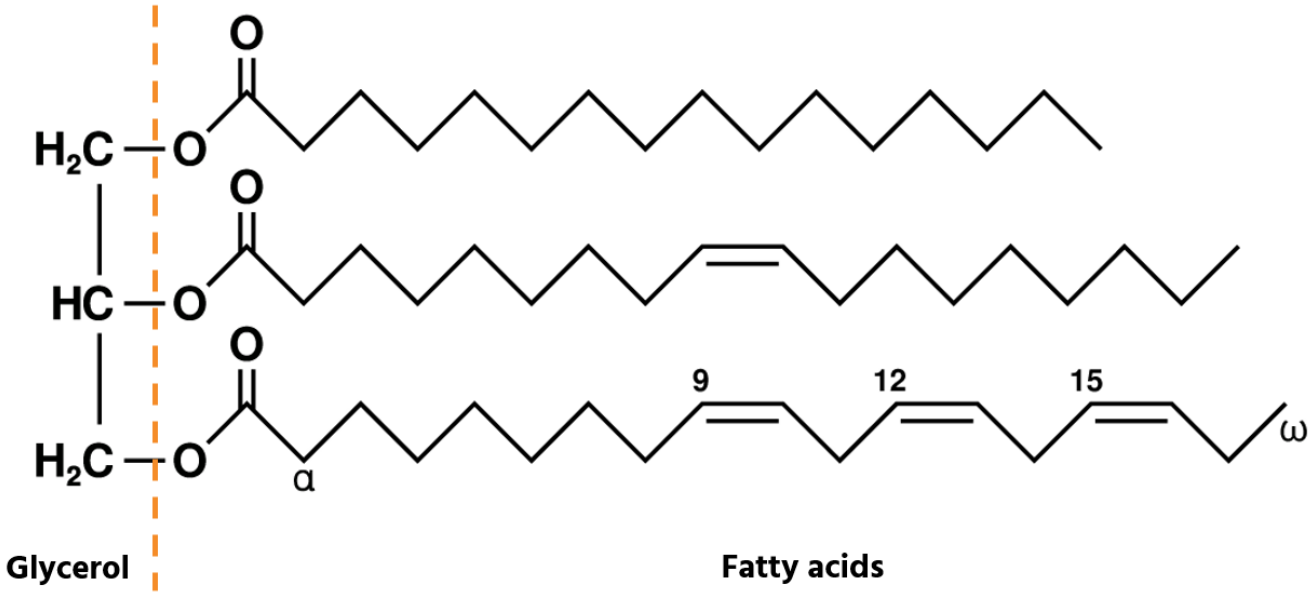
Structural model of C12:0



Molecular structure of triglyceride



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2. FATTY ACID UNITS

FAT AND FATTY ACIDS

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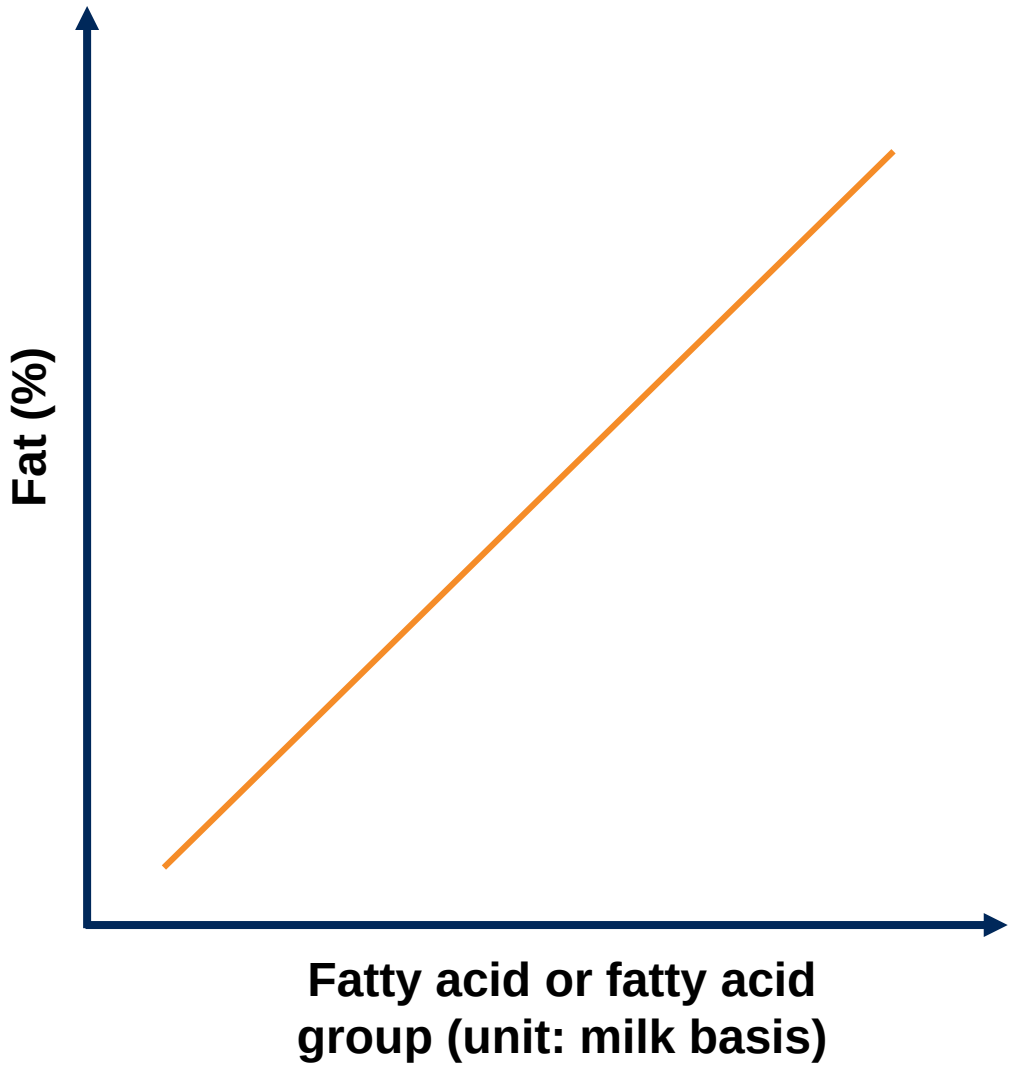

→ **Natural correlation between fat and fatty acids**

Fat (%)	Fatty acid (group), milk basis	Fatty acid (group), fat basis
1	0.1	10.52
2	0.2	10.52
3	0.3	10.52
4	0.4	10.52

according to Eskildsen et al., 2014

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FATTY ACIDS – UNITS
MILK BASIS VS FAT BASIS

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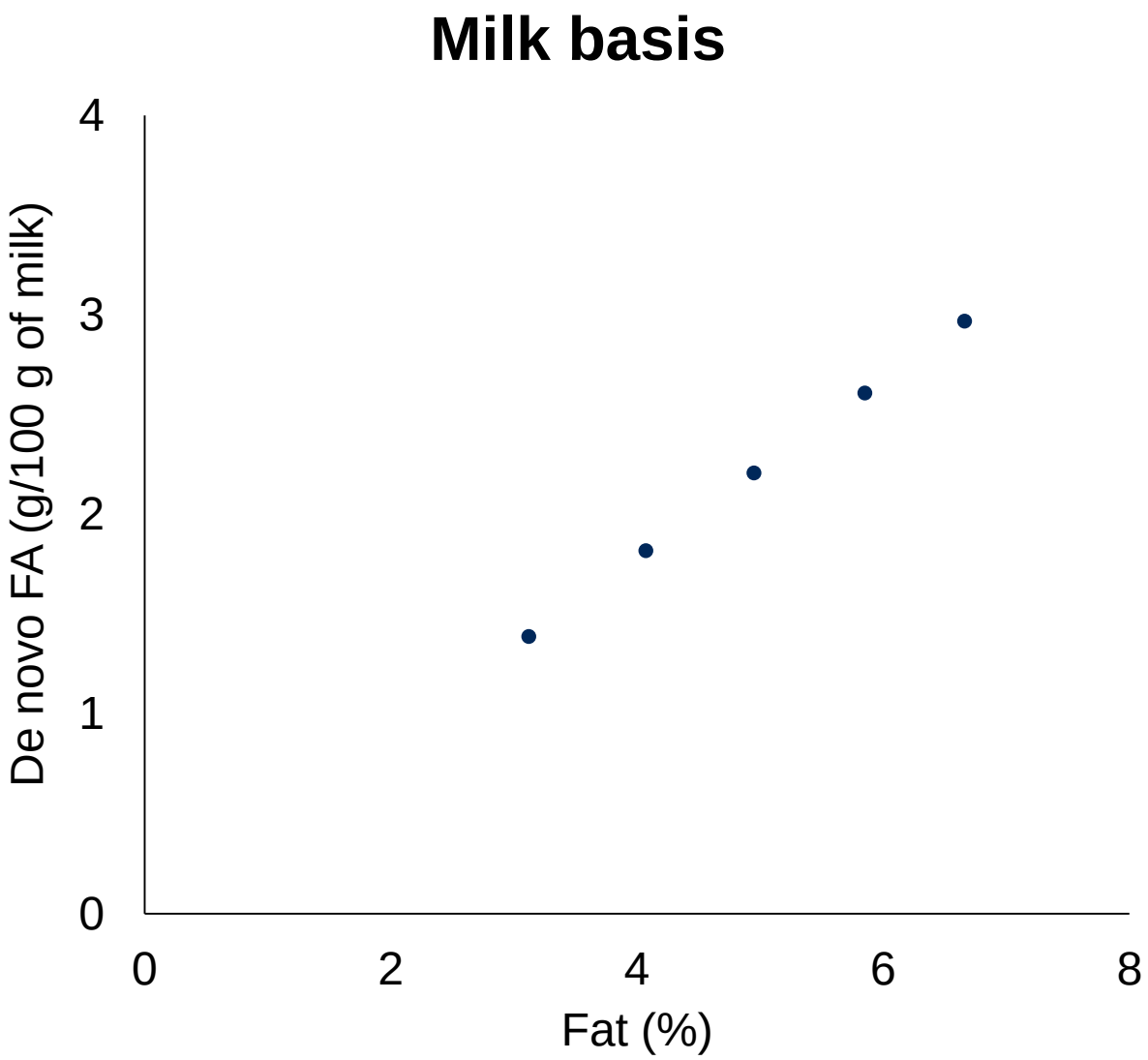

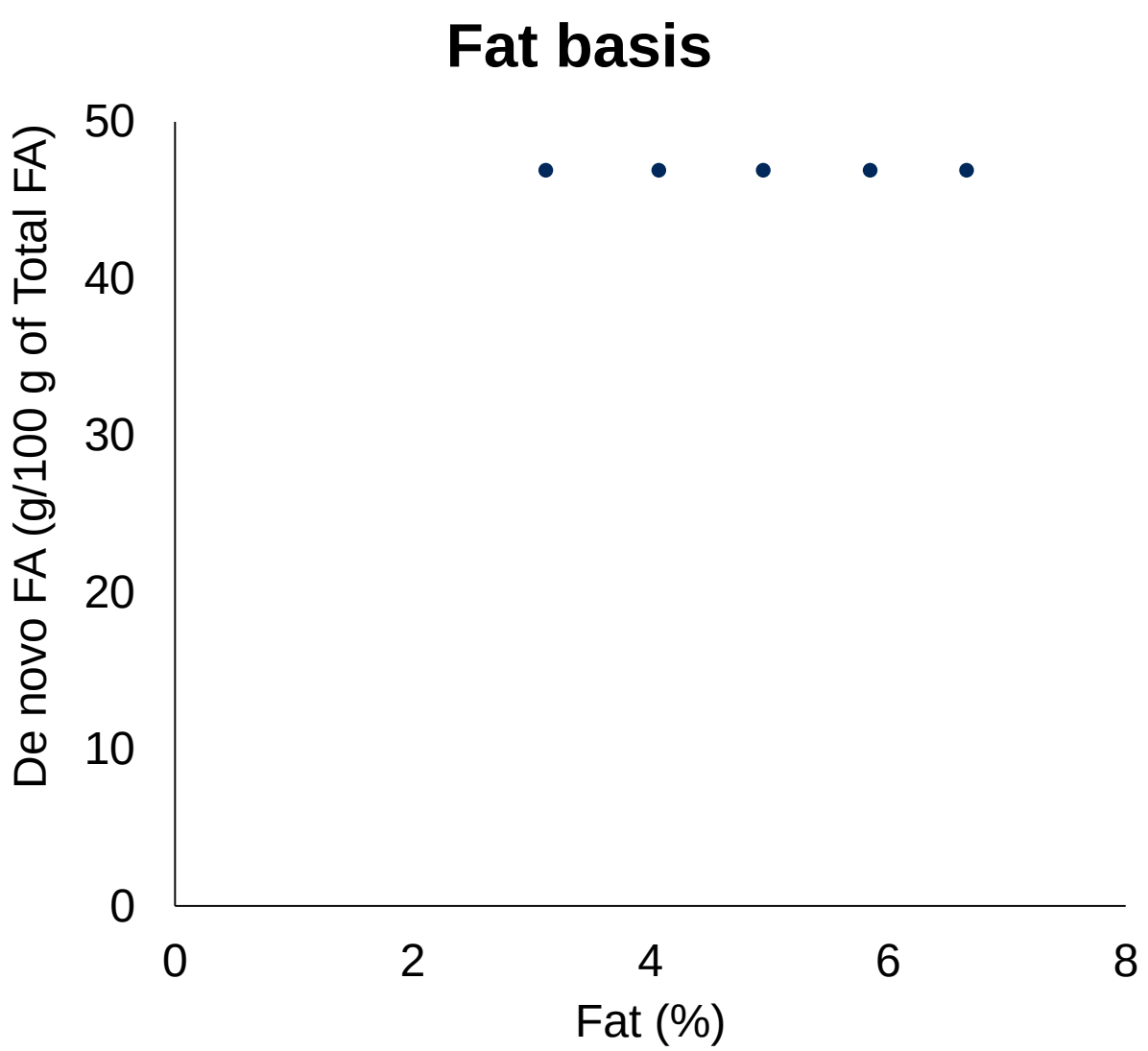

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3. FATTY ACID ORIGIN PACKAGE

FATTY ACID ORIGIN GROUPING

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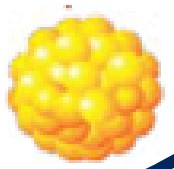
Fatty acid group:	De novo	Mixed	Preformed
Fatty acids:	≤ *C14*	*C16*	*C15* *C17* ≥*C18*
Origin of fatty acids:	Synthesized in the mammary gland	de novo or preformed	Come from feed or from body reserves



CALIBRATION DEVELOPMENT

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FOSS standards

- Calibrations/prediction models based on natural material (i.e. raw milk) only
- Development of global models
  - Samples from around the globe included (reference and spectra data) → robustness
  - Variation in cow breeds and different seasons covered → robustness
- Regular updates of models (as industry evolves)
- Variation of results in samples more important than number of samples

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VALIDATION OF DEVELOPED MODELS

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ANALYTICS BEYOND MEASURE

- Calibration development done on separate sample set; no calibration samples included!
- 303 samples available in total: 84 individual cow milk samples, 219 bulk tank/pen samples
- Geographical areas: 3 sites in USA, 2 in Canada, one each in Denmark, Germany, Japan, the Netherlands, Norway, Spain, Sweden, and the UK

VALIDATION – RESULTS ON BULK TANK/PEN SAMPLES

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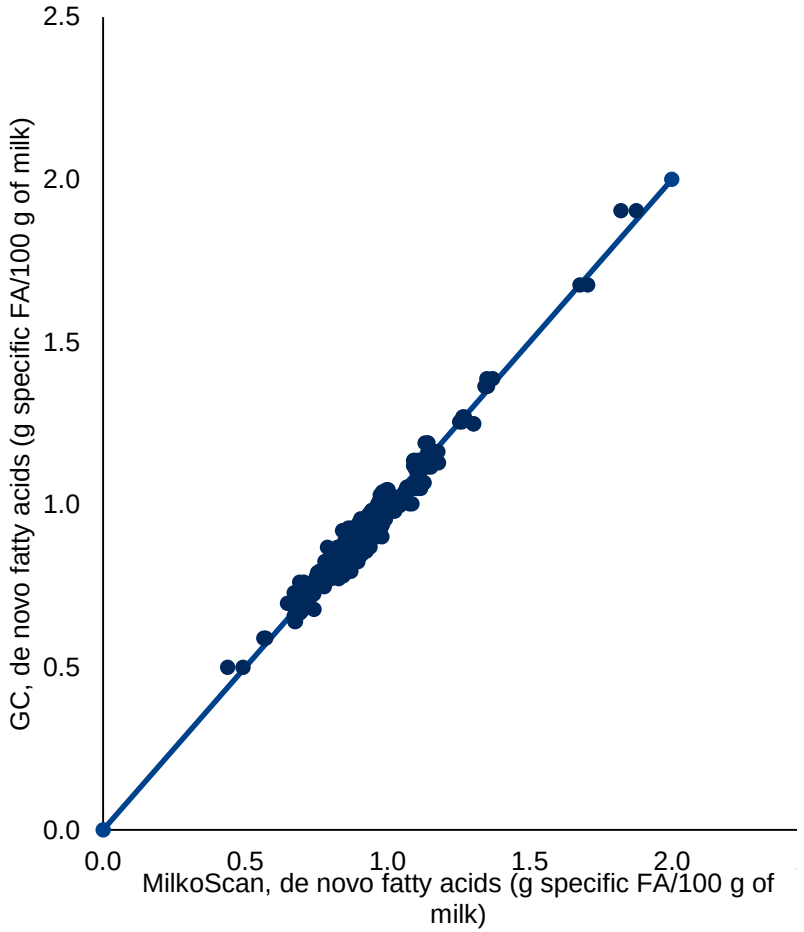
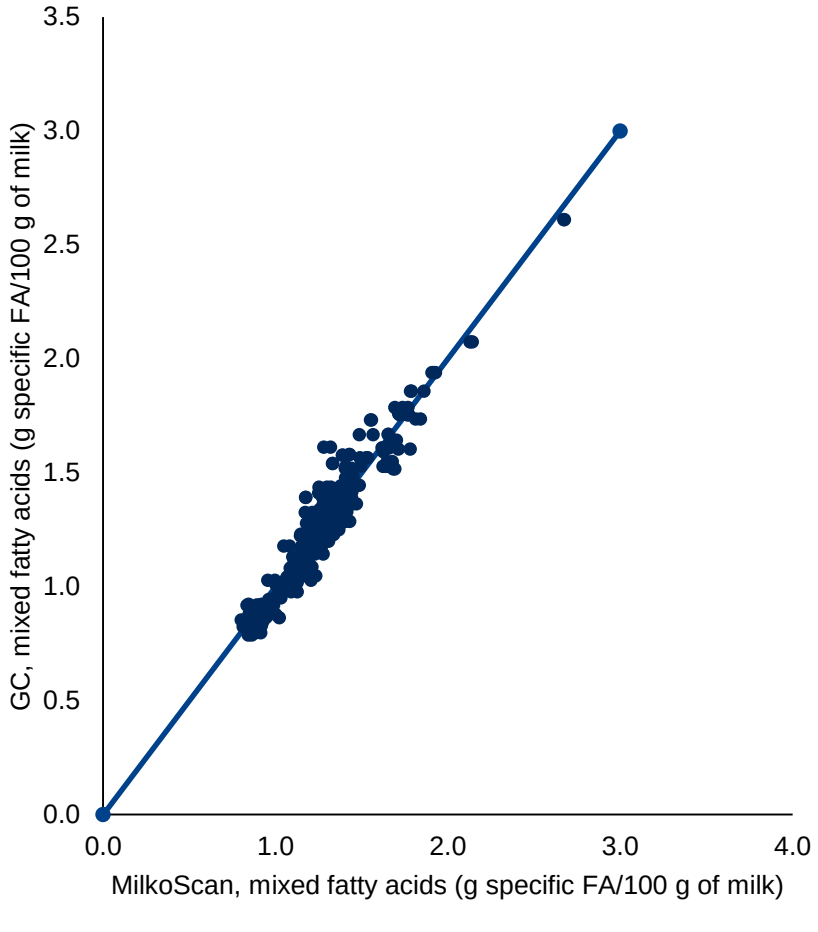
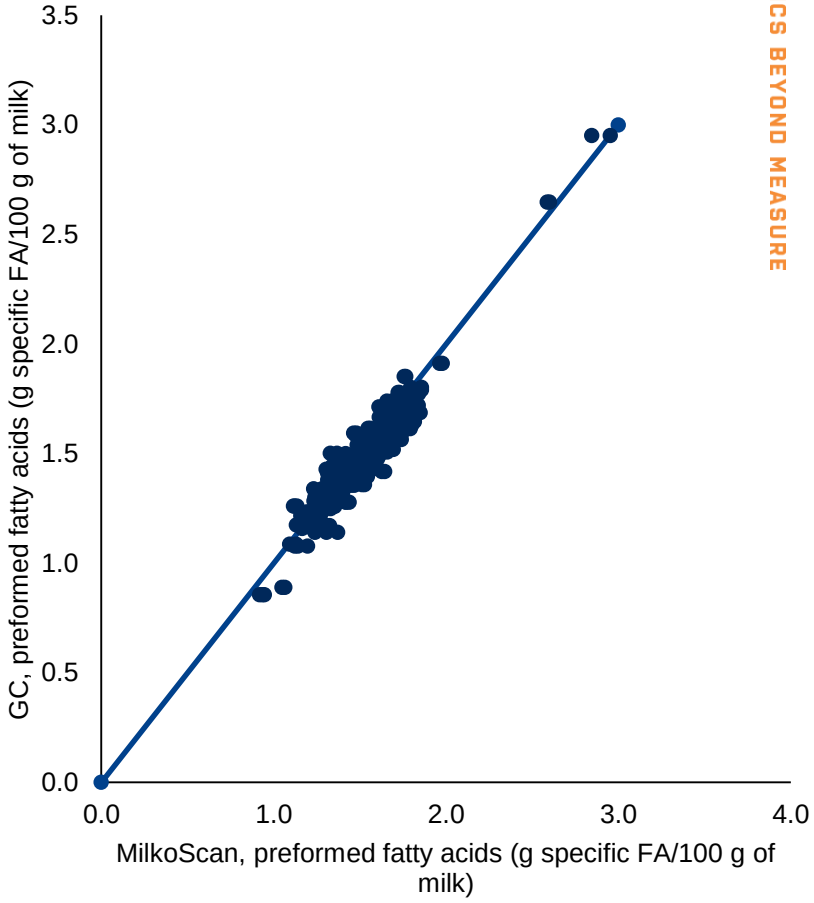
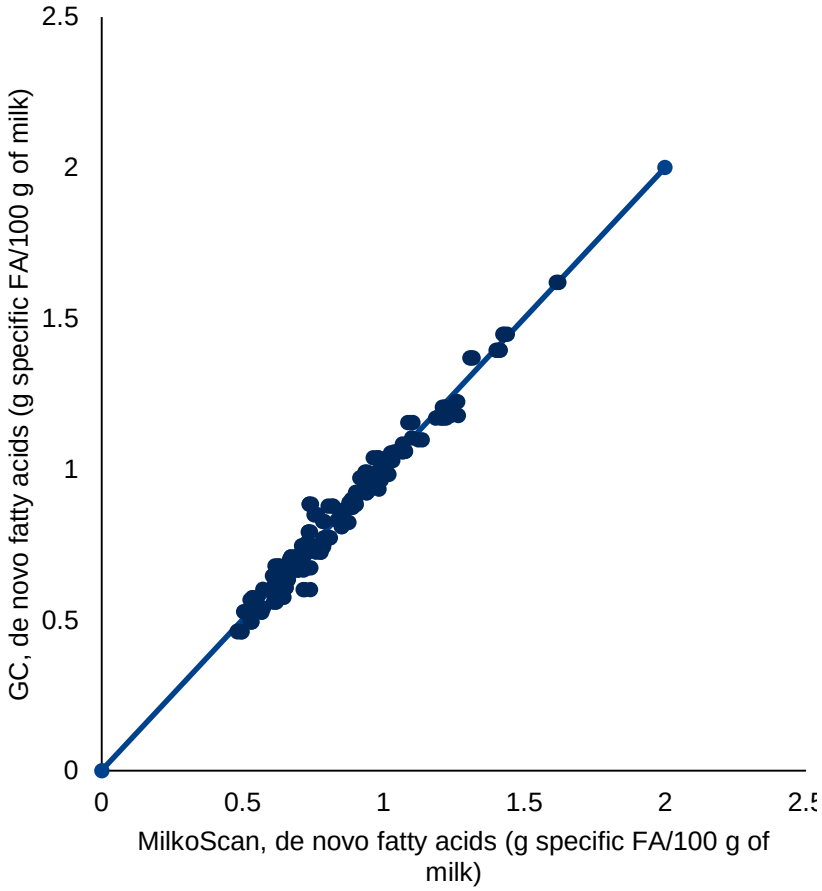


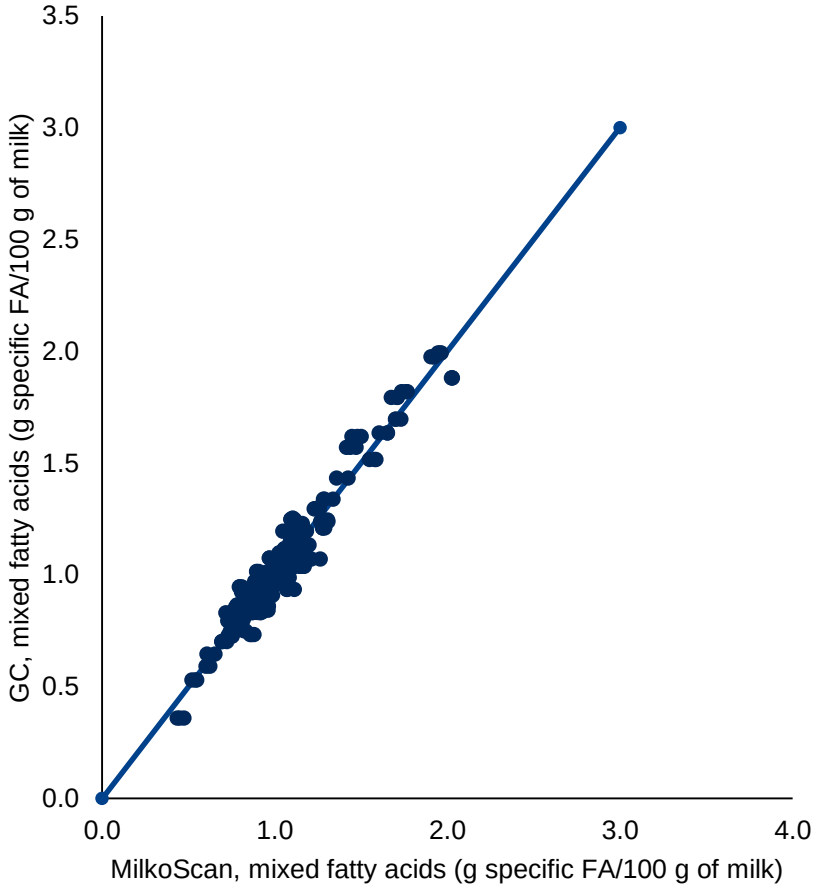


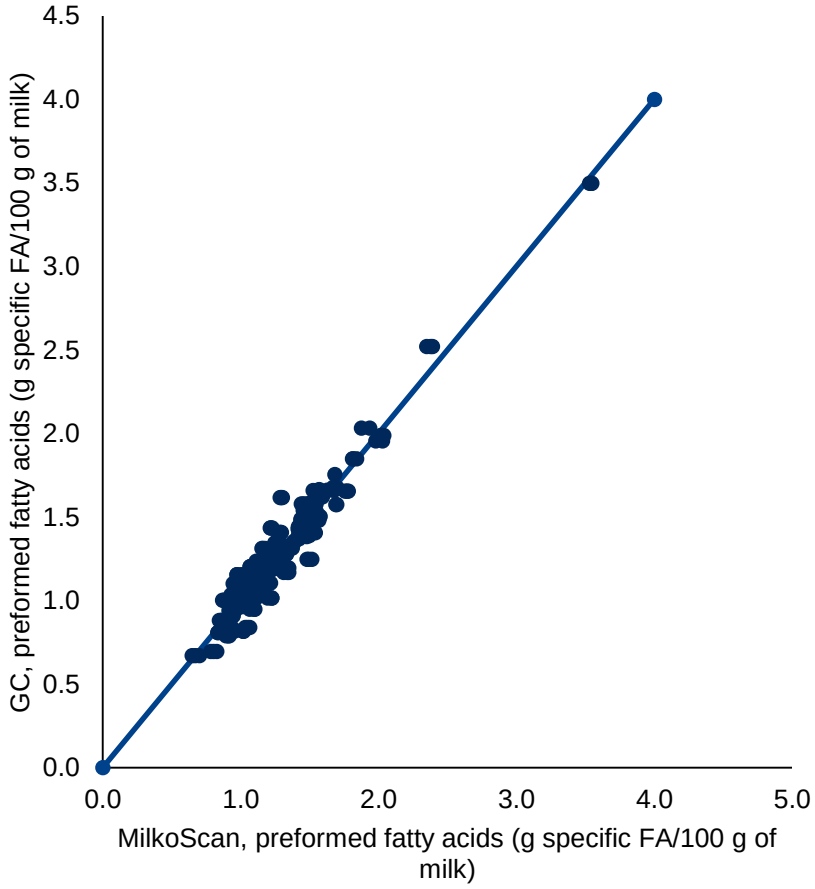


ANALYTICS BEYOND MEASURE

VALIDATION – RESULTS ON INDIVIDUAL COW MILK SAMPLES







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4. QUALITY ASSURANCE

QUALITY ASSURANCE PROCEDURE

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1. Regular standardisation of MilkoScan
2. Adjustment against reference method or based on calibration samples
3. Monitoring of instrument based on check samples

FATTY ACIDS – CALIBRATION SAMPLES

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Unit: g FA/100 g milk

Fatty acid origin groups		
De novo	Mixed	Preformed
1.39	0.69	1.04
1.82	0.90	1.36
2.21	1.09	1.65
2.61	1.29	
2.97		

No true FA variation – Not fit for purpose

Milk

$$\frac{\text{...ic Fatty Acid}}{100g\ Total\ Fatty\ Acids}$$

Fat basis

% fat
3.12
4.07
4.95
5.85
6.66

Unit: g FA/100 g TFA

Fatty acid origin groups		
De novo	Mixed	Preformed
46.9	23.2	35.2
46.9	23.2	35.2
46.9	23.2	35.2
46.9	23.2	35.2
46.9	23.2	35.2

IDF 447:2010

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EXAMPLE

DEVELOPMENT OF CALIBRATION SAMPLES

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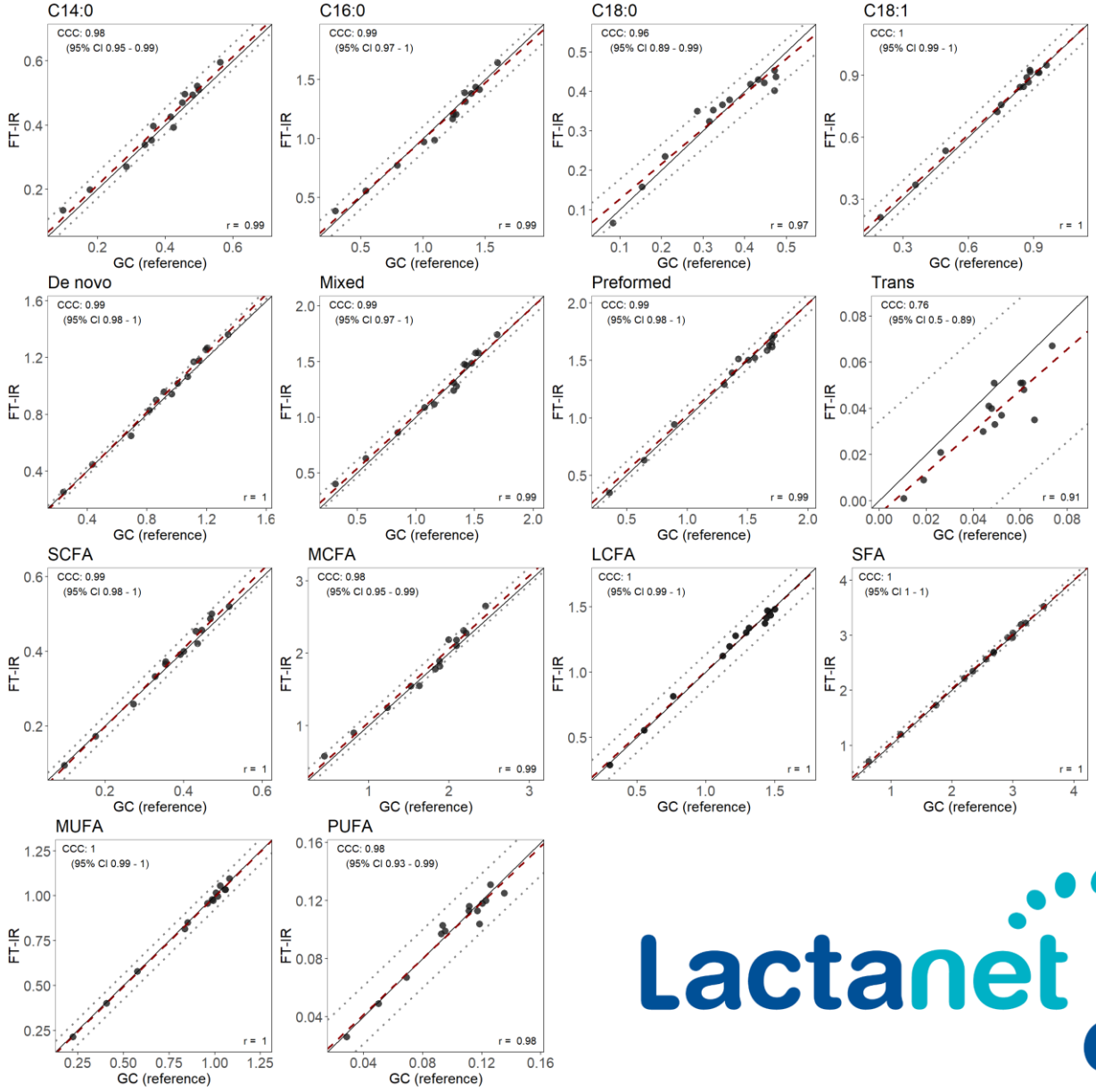
- Bulk tank milk from different farms
- 14 samples offering true variation in FA composition

→ Excellent correlation between FTIR and reference results



Lactanet

ANALYTICS BEYOND MEASURE

FATTY ACID ORIGIN – DATA COLLECTION

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- Each month 14 calibration samples with GC results were prepared
  - Natural milk from mother nature (Adulteration not recommended)
  - Samples selected to have as wide a matrix variation as possible
  - Minimal covariance between different fatty acids

- Sample sets send to multiple instrument
  - MSC 7 (3 instruments – 2 different sites)
  - FT plus (3 instruments – Same site)

- First sample set tested end of March 2019 and last sample set tested early August (5 sets in total)
  - Time period between first and last adjustment is 4.5 months

TYPE OF ADJUSTMENTS – OPTIMAL ACCURACY

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- 70 samples collected and scanned over 4.5 months period
- 5 sets of 14 calibration samples

- **Experiment:**

  - Adjustments of Slope/Intercept every set (Months)

  - One adjustment used over entire time period

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TYPE OF ADJUSTMENTS

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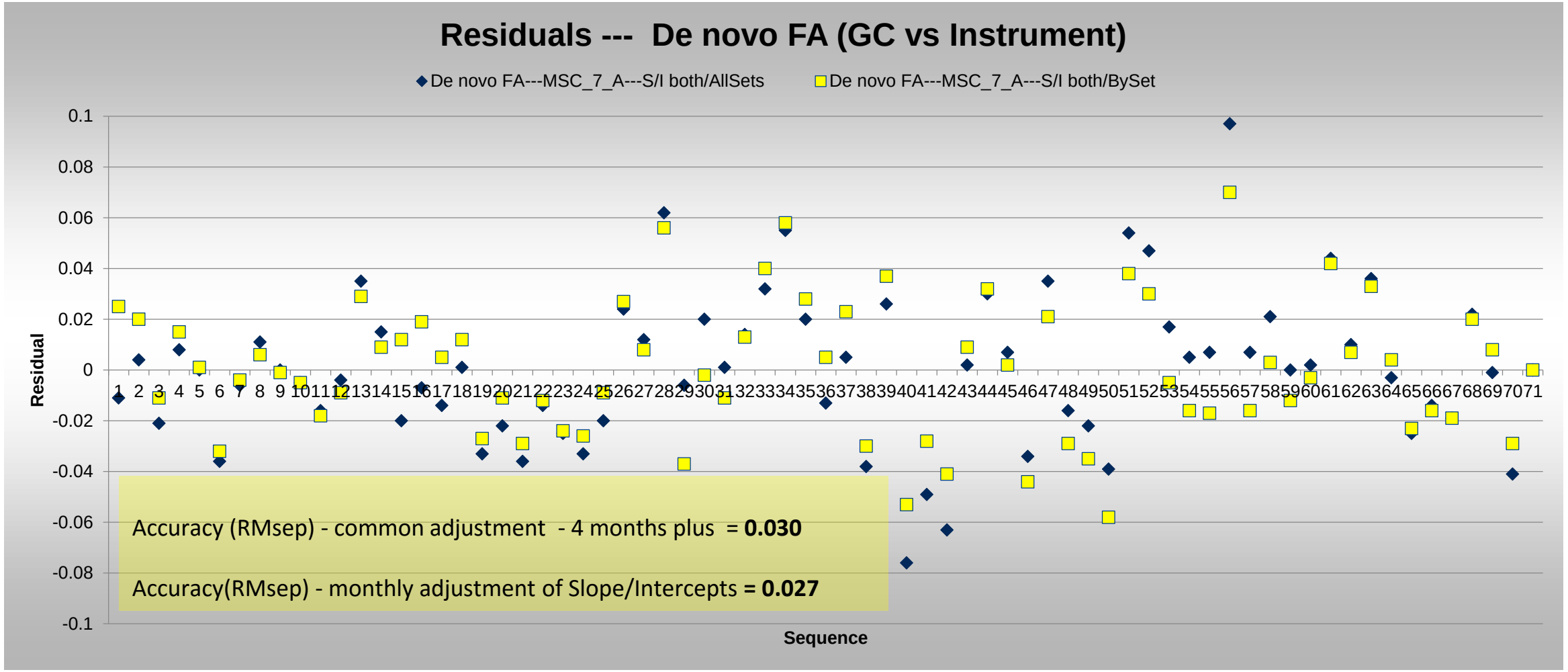

ANALYTICS BEYOND MEASURE

TYPE OF ADJUSTMENTS

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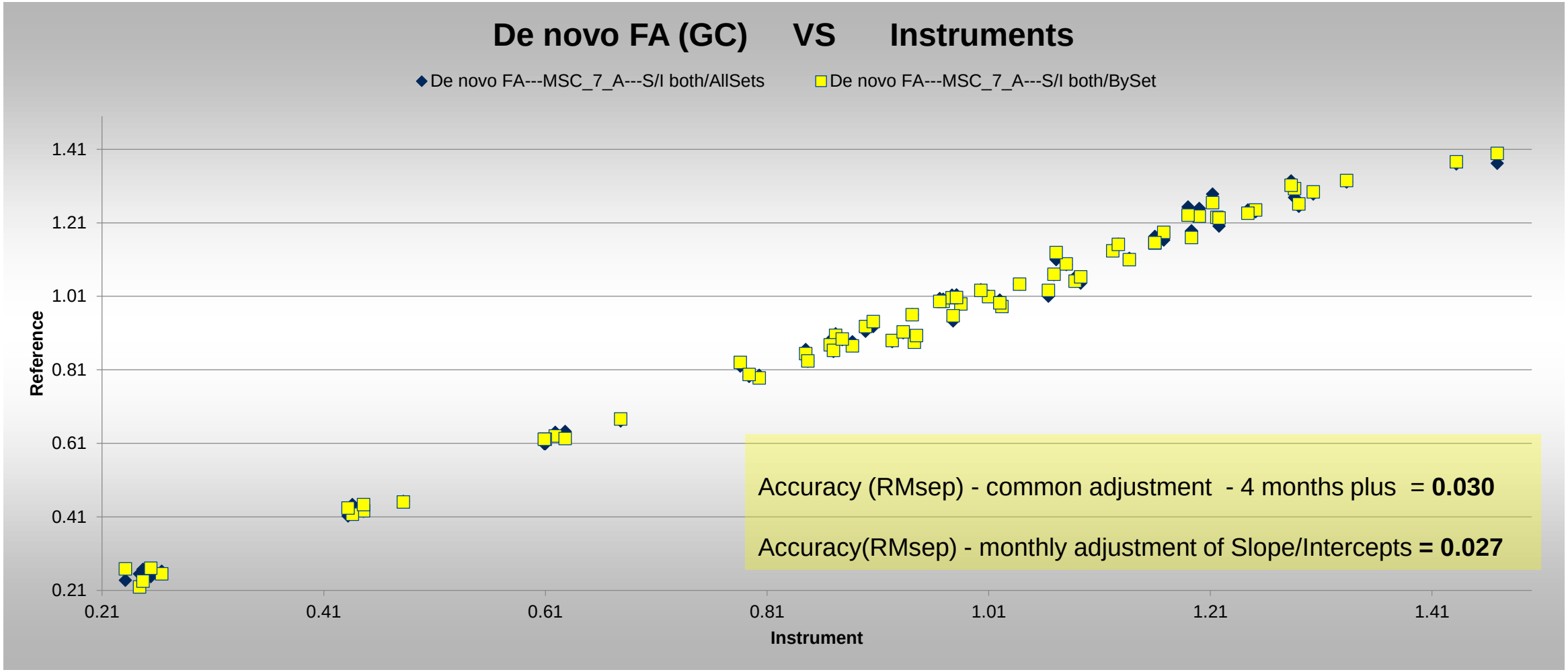

ANALYTICS BEYOND MEASURE

TYPE OF ADJUSTMENTS

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- Conclusions:
  - A single adjustment can be used for a long period of time
  - No need to adjust the calibration monthly
  - Developing guidelines for how best to manage calibration adjustments

TRANSFERABILITY – INSTRUMENT VS INSTRUMENT

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- The same 5 sets of calibration samples have been tested on 6 instruments
- Instruments known as ***MSC_7_A*** is considered the master instrument
- The master instrument is calibrated against reference chemistry (GC lab data)
- The other instruments are calibrated to the master instrument
- The room mean standard deviation of the differences has been calculated as follows

TRANSFERABILITY – INSTRUMENT VS INSTRUMENT

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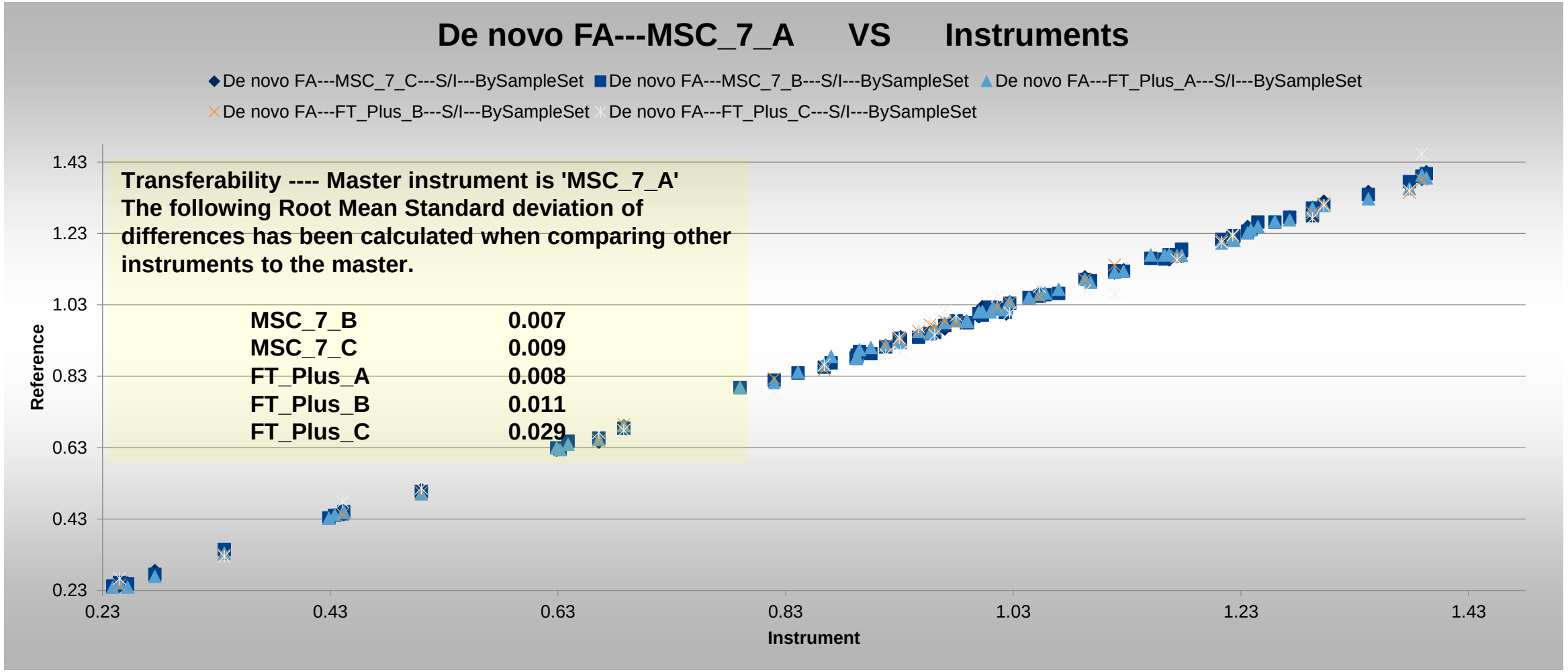

ANALYTICS BEYOND MEASURE

TRANSFERABILITY – INSTRUMENT VS INSTRUMENT

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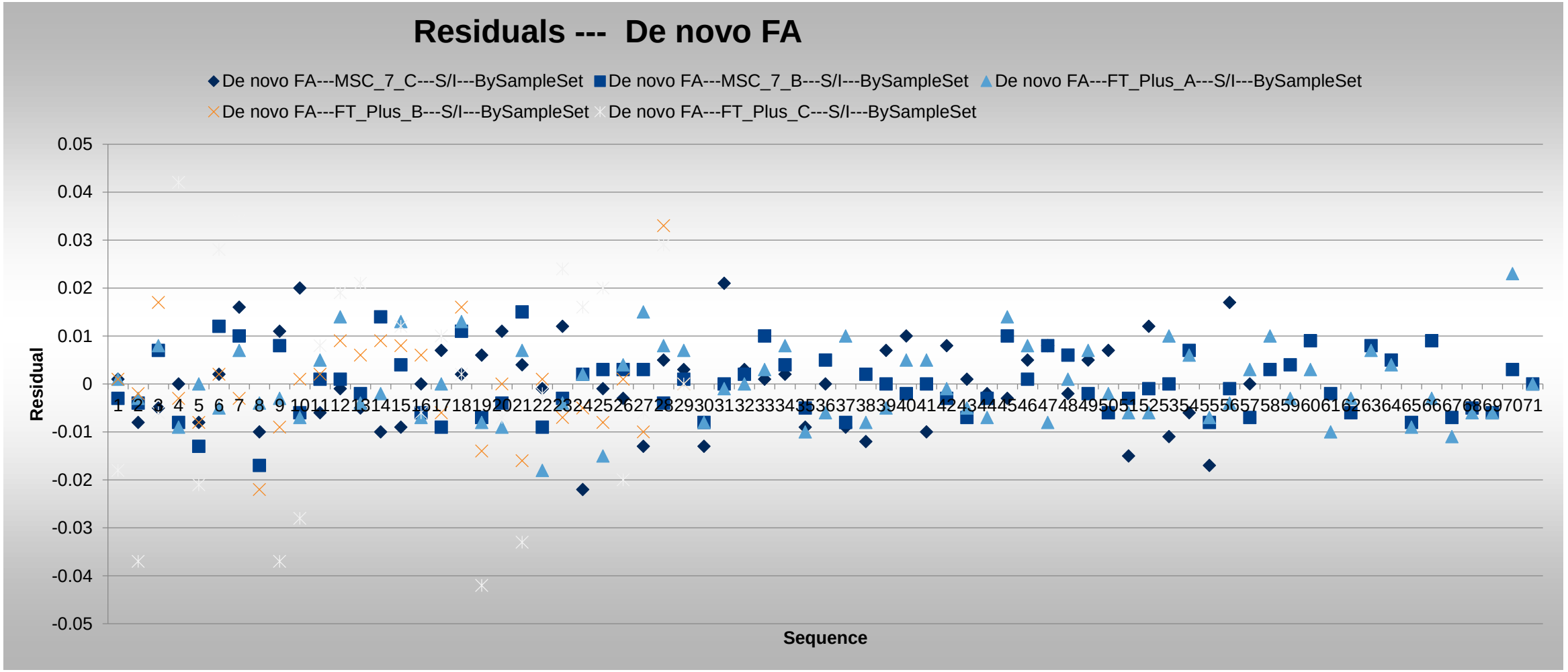

ANALYTICS BEYOND MEASURE

TRANSFERABILITY – INSTRUMENT VS INSTRUMENT

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ANALYTICS BEYOND MEASURE

- **The following Root Mean Standard deviation of differences has been calculated when comparing other instruments to the master.**

MSC_7_B	0.007
MSC_7_C	0.009
FT_Plus_A	0.008
FT_Plus_B	0.011
FT_Plus_C	0.029

CONCLUSIONS ON TRANSFERABILITY – INSTRUMENT VS INSTRUMENT

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- The instrument to instrument agreement is significantly better than comparing to reference data (GC lab data)
- Satellite instruments can be calibrated to a master instrument.
- Master instrument must be calibrated against reference data (GC lab data)

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5. POTENTIAL APPLICATIONS

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ANALYTICS BEYOND MEASURE

HERD LEVEL

FAT AND FATTY ACID PROFILE

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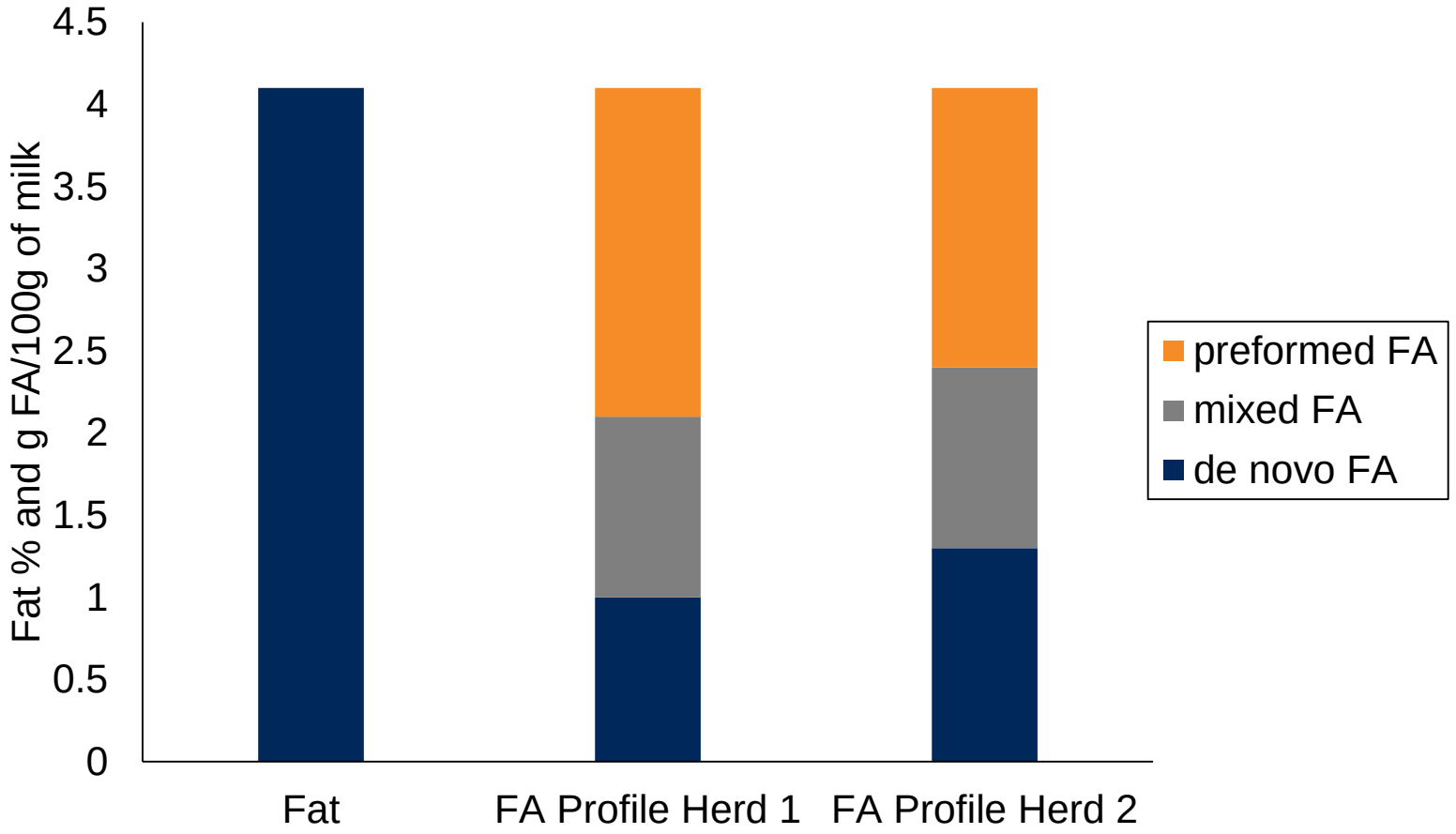
ANALYTICS BEYOND MEASURE

Fat naturally produced or through fat supplements?



REAL LIFE EXAMPLE
FAT AND PROTEIN VS DE NOVO FA

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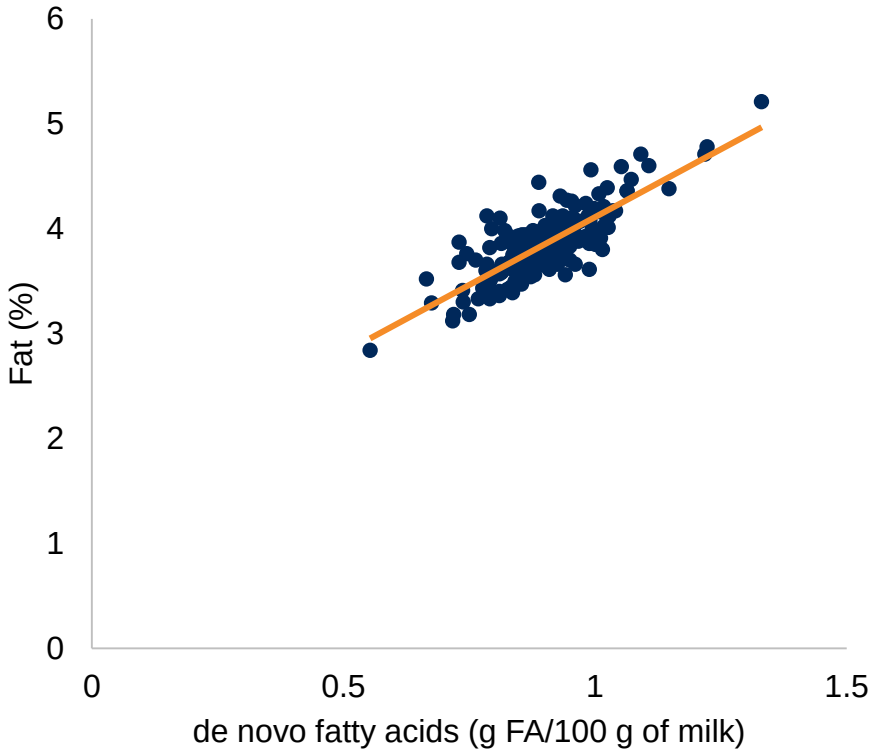
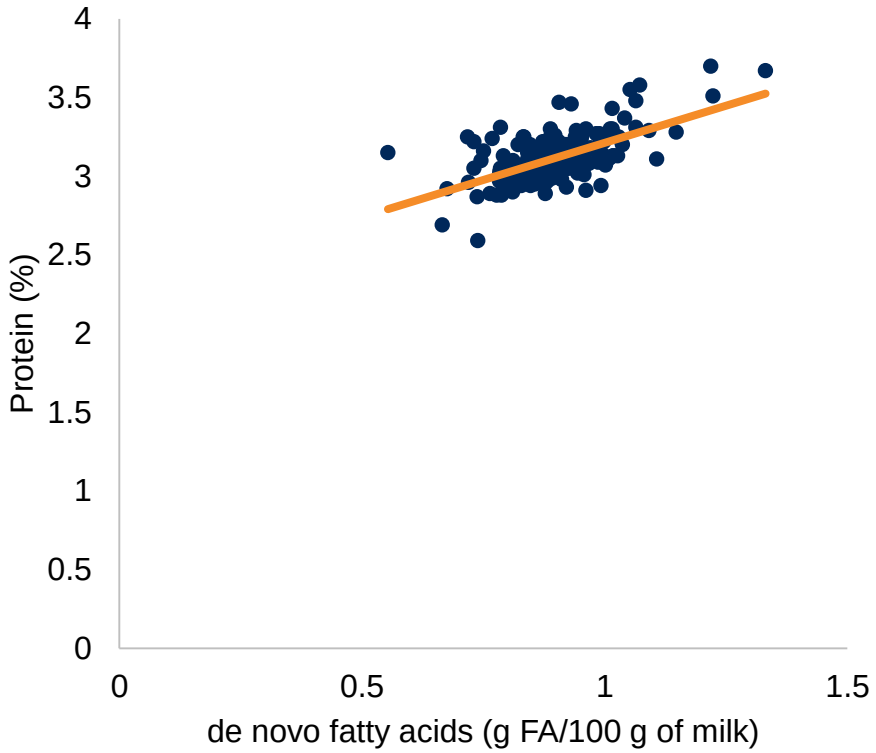




→ 200 dairy farms,
1 test per farm

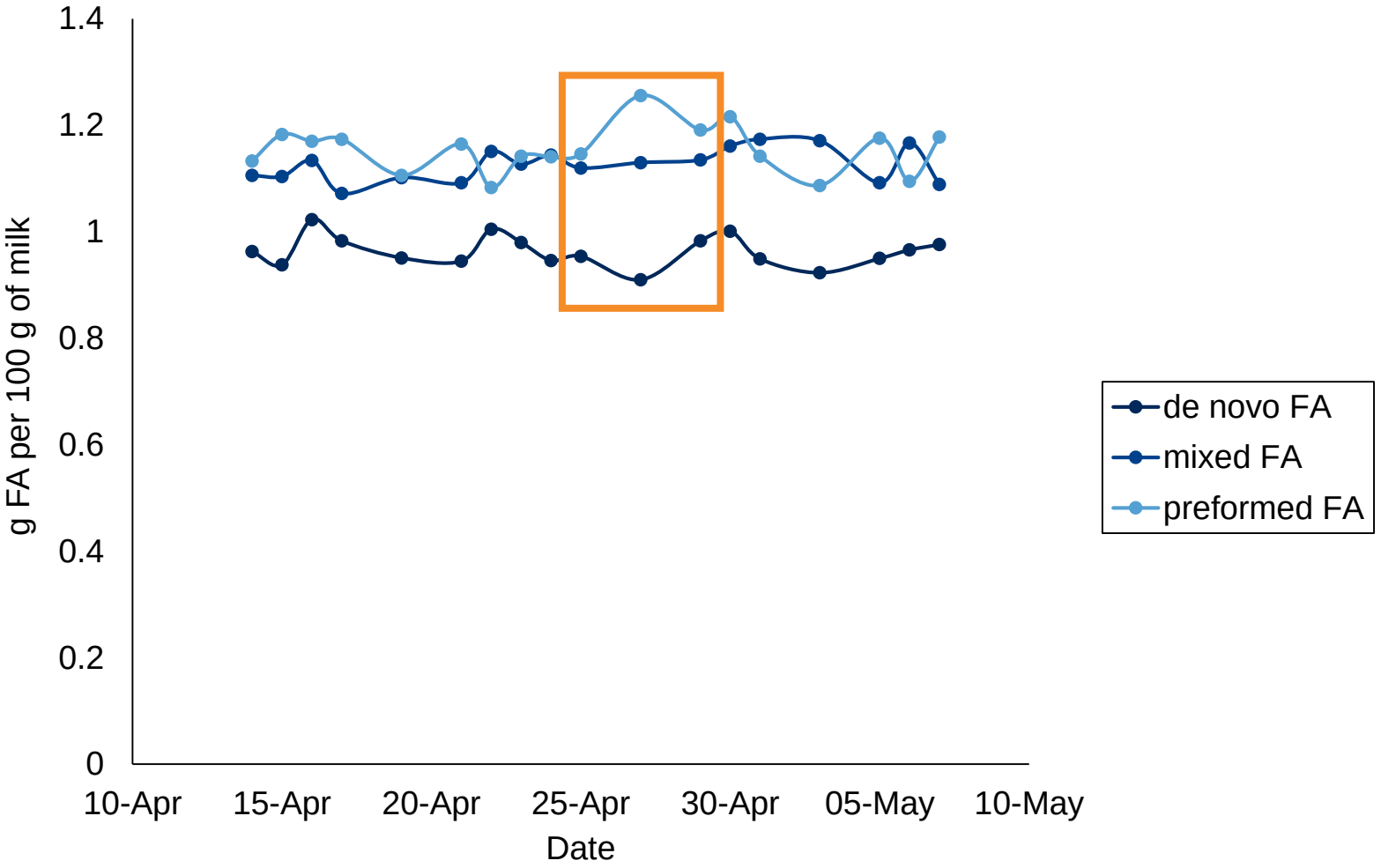
ANALYTICS BEYOND MEASURE

High **fat %** associated with high de novo contents
→ increased function of rumen as well as production of volatile fatty acids

High **protein %** associated with high de novo contents
→ Increased microbial fermentation as well as microbial protein synthesis

→ Opportunity for dairy farmers to increase revenue and profit

REAL LIFE EXAMPLE: HERD MANAGEMENT



What happened?

- Cows started mobilizing
- De novo synthesis went down

Reason?

Change in silage quality (more fibre/less digestible)

Idea:

Changes in fatty acid profile can be noticed a few days before milk or fat yield start to decrease

→ Dairy farmer can react EARLIER and save $$$

By courtesy of Debora Santschi

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COW LEVEL

INDIVIDUAL COW FA DATA – EXAMPLE

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Pen or group A

	Fatty acid group		
FA level	De novo	Mixed	Preformed
Low	7 (35%)	1 (5%)	0 (0%)
Medium	12 (60%)	18 (90%)	8 (40%)
High	1 (5%)	1 (5%)	12 (60%)

Pen or group B

	Fatty acid group		
FA level	De novo	Mixed	Preformed
Low	6 (15%)	2 (5%)	23 (59%)
Medium	14 (36%)	26 (67%)	9 (23%)
High	19 (49%)	11 (28%)	7 (18%)

DOCUMENTATION

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A WHITE PAPER FROM FOSS:

Fatty acid profiling according to origin for optimising feeding and management of dairy cows – a new approach

ANALYTICS BEYOND MEASURE

By: Dr. Daniel Schwarz
fossanalytics.com
November 2018, GB

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A WHITE PAPER FROM FOSS:

The new FOSS fatty acid origin package – Basics behind the prediction models

ANALYTICS BEYOND MEASURE

By: Dr. Daniel Schwarz, Maria Rosenberg Bak and Dr. Per Waaben Hansen, FOSS Denmark
fossanalytics.com • November 2018, GB

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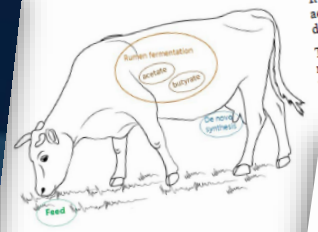
Application Note

AN 5465
Rev. 2

MilkoScan™ 7 RM/ FT+ / FT 6000

Fatty Acid Origin Package



It is well-known that changes in both milk fatty acid composition and total fat content occur depending on the cow's diet.

This Application Note describes three prediction models of fatty acid groups, which are based on the actual origin of fatty acids: de novo, mixed and performed.

Application Note 5465 / Rev. 2

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Fatty Acid Origin

A package of prediction models to help dairy farmers optimize dairy-herd feeding.

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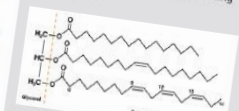
Milk fatty acid composition is clearly associated with the diet of dairy cows which, in turn, opens up the opportunity to improve herd efficiency via more effective feeding strategies. Specifically, the milk fatty acid origin profile of bulk tank samples allows effective evaluation of the feeding status of the entire lactating herd for improved fat and protein content in milk deliveries.

The Fatty Acid Origin package allows you to determine the fraction between the fatty acids according to their origin as guideline for feeding programs and management of cows. It consists of three prediction models: 1) de novo fatty acids, 2) mixed fatty acids, and 3) preformed fatty acids. The specific fatty acids included in each prediction model are shown in Fig 1. overleaf.

The new models were developed based on full spectra data and gas chromatography reference data from multiple countries on three continents (Europe, North America, Asia). All data used originated from raw milk samples only (both bulk tank and individual cow milk samples) to reflect routine conditions in milk-testing laboratories.

Fat and fatty acids

- Fat in milk mainly appears in form of a complex combination of lipids called triglycerides
- Triglycerides consist of esters of three different fatty acids with one molecule of glycerol each (see molecular structure)
- Fat content as well as the fatty acid composition are associated with animal-related factors and the management and feeding of the animal



A MESSAGE TO TAKE HOME

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ANALYTICS BEYOND MEASURE

- Accurate, reliable, transferable, and stable generation of milk fatty acid profiles
- New possibilities to improve feeding management
- The power of co-creation

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