Interest, Recording and Possible Use of New Phenotypes from Fine Milk Composition

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Milk Composition

- Until recently 5 major constituents
  - Milk fat, protein, urea nitrogen, lactose and somatic cell count
- However
  - Milk is a very complex substance with large number of constituents
  - Some major constituents themselves complex groupings of minor constituents

Fine Milk Composition

- Milk fat
  - Fatty acids mostly as triglycerides
  - Free fatty acids
- Milk protein
  - Caseins
  - α-lactalbumins
  - β-lactoglobulins
  - Other minor proteins (e.g., lactoferrin)
- Other minor constituents
  - Minerals
  - Vitamins
  - ....

Milk Composition

- From an animal breeder's standpoint
  - Breed differences and genetic variability exist, not only for major milk constituents
  - Also recently shown for
    - Fatty acids
    - Lactoferrin
    - Major minerals
- Complexity of milk composition
  - Very useful to define new phenotypes describing environment-animal-product

Defining (New) Phenotypes

- Animal selection, as well as herd management
  - Based on the precise assessment of important traits
- New phenotypes can be defined
  - Better and finer knowledge of milk composition
- Phenotypes for and in at least four areas:
  - Herd management,
  - Environment,
  - Animal health, and
  - Milk quality

Defining (New) Phenotypes

- Definition of phenotypes,
  milk constituents used as:
  - Direct measure of considered traits of interest
  - Indirect indicator of traits of interest
- Knowledge of these traits
  - Positive impact on sustainability of milk production
  - Because new traits open new opportunities for selection and management

Current Traits

- Fat content
- Protein content
- Urea
- Lactose
- Casein
- Free fatty acids

Milk payment + milk recording

Milk recording

Few milk recording

➔ All these predicted by **Mid InfraRed Spectrometry (MIR)**

Why Mid-Infrared?

- **Advantages of MIR spectrometry**
  - Fast and cheap
  - No destructive method
  - Environmentally friendly
- **Because of these advantages**
  - MIR largely used by milk labs to quantify the major constituents of milk
  - However generates more than the currently available traits

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    $\Rightarrow$ **MIR spectrum**

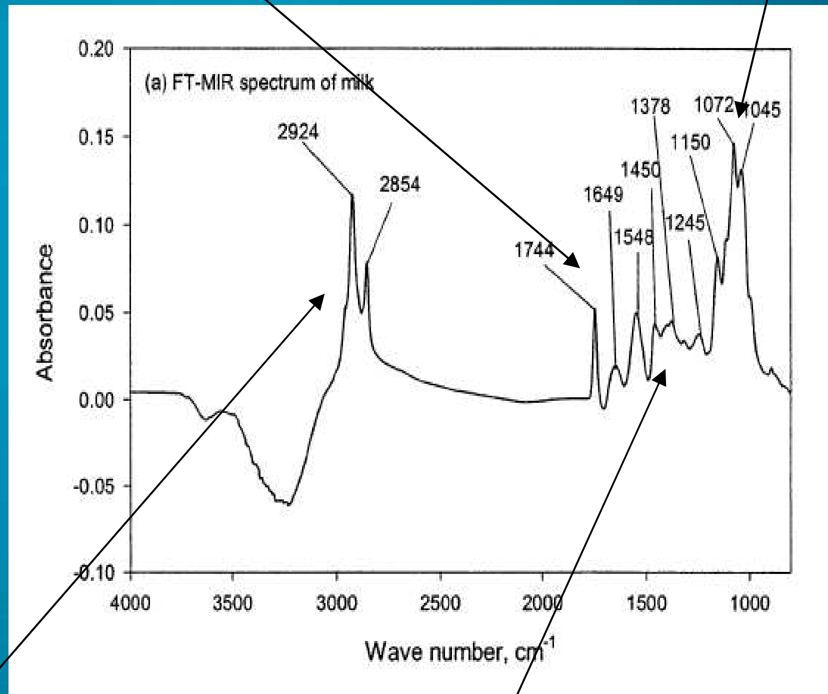
MIR Spectrum

- **MIR spectrum:**

absorptions of infrared at frequencies correlated to the vibrations of specific chemical bonds within a molecule

1700 – 1500 cm^{-1} : N-H

1200 – 900 cm-1 : C-O



(Sivakesava and Irudayaraj, 2002)

3000-2800 cm^{-1}: C-H

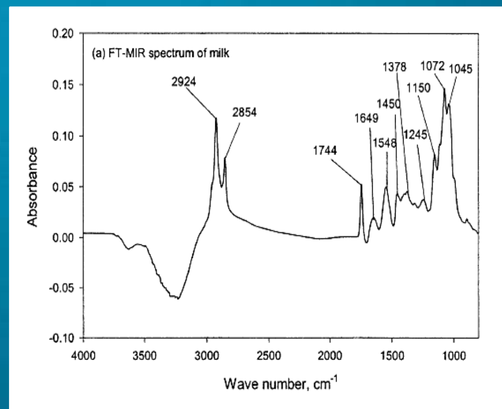
1450-1200 cm-1 : COOH

How It works!

Collection of milk samples

(Foss, 2008)

MIR spectrometer



Raw data = Spectra

Calibration equations

Predictions:

- Fat content
- Protein content
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Development of new equations

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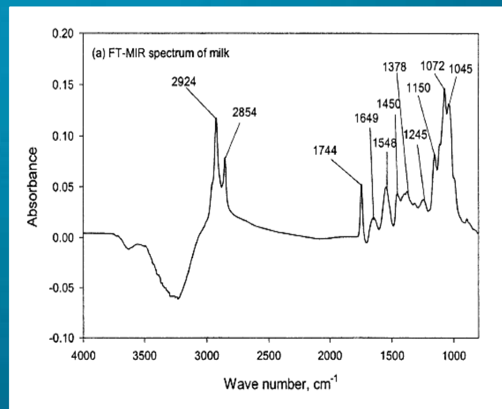

Raw data = Spectra

New Predictions:

- Fatty acids
- …

Calibration

- **Key issue: calibration**
  - ❑ **Need for reference samples**
  - ❑ **By experience should be as diverse as possible**
    - **Breeds, herds, production systems, MIR spectrometers**
  - ❑ **Highly specialized field in itself**
- **Will not go more into details**
  - ❑ **Please refer to different papers by Hélène Soyeurt**

Recording Phenotypes

MIR spectrum

Animal Health

Fatty acids, minerals, lactoferrin, lactose, β-hydroxybutyrate, acetone,…

Environment

Fatty acids, methane emission through fatty acid contents, urea

Herd Management

Urea, fat, protein, lactose,…

Biodiversity

Changes in milk composition

Nutritional Quality of Milk

Fatty acids, lactoferrin, minerals,…

Hygienic Quality of Milk

Antibiotics, somatic cells,… (less important for milk recording)

Technological Quality of Milk

Traits related to cheese making (casein, dornic acidity, coagulation time,..),…

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Environment

Fatty acids, methane emission

GREENHOUSEMILK

Herd Management

Urea, fat, protein, lactose,…

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Few examples…

Fatty Acids (FA)

- Recent studies confirmed the ability of MIR to predict FA in milk:
  - Soyeurt et al. (2008, 2009), Rutten et al. (2009)
- Lower ability to predict FA content in fat
  - Results presented here therefore based on FA content in milk
- New results obtained in the RobustMilk project (www.robustmilk.eu)
  - Multi-breed, multiple countries and multiple production systems

Fatty Acids

- Study presented in details at ADSA meeting 2010

Constituent (% in milk)	N	Mean	SD	RPD	SECV
Saturated FA	496	2.40	0.80	15.7	0.0513
Monounsaturated FA	491	1.06	0.37	8.9	0.0411
Polyunsaturated FA	499	0.16	0.05	2.6	0.0204
Unsaturated FA	492	1.22	0.41	9.6	0.0428
Short chain FA	486	0.31	0.11	6.7	0.0165
Medium chain FA	496	1.78	0.60	6.5	0.0928
Long chain FA	495	1.52	0.57	6.5	0.0875

SECV = standard error of cross-validation;
RPD = ratio of standard deviation of reference values to the standard error of cross-validation

Soyeurt, H., F. Dehareng, N. Gengler, S. McParland, E. Wall, D.P. Berry, M. Coffey, and P. Dardenne. 2010. J. Dairy. Sci. Submitted.

Minerals

- First results were published by Soyeurt et al., 2009

mg/l milk	N	Mean	SD	SECV	RPD
Ca	87	1,333	260	95	2.74
K	61	1,336	168	136	1.24
Mg	61	110	18	11	1.68
Na	87	403	107	64	1.68
P	87	1,093	127	50	2.54

SECV = standard error of cross-validation;
RPD = ratio of standard deviation of reference values to the standard error of cross-validation

- Current study confirmed these results with a larger dataset (more than 100 samples)

Lactoferrin

mg/l milk	N	Mean	SD	SECV	RPD
Lactoferrin	57	253	206	86	2.39

SECV = standard error of cross-validation;
RPD = the ratio of standard deviation of reference values to the standard error of cross-validation

- **Milk glycoprotein involved in the immume system defenses**
- **Premilary results published in 2007**
- **Validation in the RobustMilk project (www.robustmilk.eu) on more than 3,000 records**

Ketone Bodies

- **Acetone: Hansen (1999) and Heuer et al. (2001)**
- **De Roos et al. (2007) studied also 2 other ketose bodies**

mMol	N	Mean	SECV	R^2cv
Acetone	1,063	0.146	0.184	0.72
β-hydroxybutyrate	1,069	0.078	0.065	0.62

R^2cv = cross-validation coefficient of determination;
SECV = standard error of cross-validation

Methane

- Chillard et al. (2009) found associations between some fatty acids and methane, explained by:
  - Common biochemical pathways between methane, acetate, and butyrate in the rumen
  - Action of dietary lipids on methane production
- However results were on few animals from feeding experience
- New European 7FP Marie-Curie project called

GREENHOUSEMILK

GREENHOUSEMILK

- Marie-Curie ITN:
  - GreenhouseMilk - Developing Genetic Tools to Mitigate the Environmental Impact of Dairy Systems
- Project leader SAC (Eileen Wall)
- Different aspects
- Contribution ULg - GxABT
  - Our group will study in detail link methane production and FA composition

GREENHOUSEMILK

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We are looking for a PhD student!

Cheese-Making

		N	Mean	SD	R^2cv	SECV
Titrable acidity (SH°/50ml)	De Marchi et al., 2009	1,063	3.26	0.43	0.81	0.25
Rennet coagulation time (min)	De Marchi et al., 2009	1,049	14.96	3.84	0.79	2.36
	Dal Zotto et al., 2008	74	15.05	3.78	0.73	0.80
pH	De Marchi et al., 2009	1,064	6.69	0.12	0.77	0.07
Titrable acidity (D°)	Colinet et al., 2010	203	16.22	2.01	0.90	0.64
Curd firmness (mm)	Dal Zotto et al., 2008	74	32.43	7.95	0.45	5.49

R^2cv = cross-validation coefficient of determination;
SECV = standard error of cross-validation

Conclusions (First Part)

- Many new phenotypes are predictable by MIR
  - Only some examples were given
- MIR is currently under used in practice
  - Most spectral data is thrown away
- Even if use of calibration equations a posteriori not feasable for farm reporting
  - Non-corrected bias from use of equations on different machines without validation
  - However for animal breeding purposes, historical data very useful

Still a lot of work to do …

“Local” Collaborators MIR

- **GxABT:**
  - Valérie Arnould – Catherine Bastin
    Frédéric Colinet – Alain Gillon – Sylvie Vanderick
- **CRA-W:**
  - Frédéric Dehareng – Pierre Dardenne
- **Comité du Lait:**
  - Didier Veselko – Emile Piraux
- **AWE:**
  - Carlo Bertozzi – Laurent Laloux – Xavier Massart

gembloux agro bio tech

Université de Liège

Use of New Traits
An example…

Use of New Traits

- **Example:**
  - **Genetic evaluation of Milk fat composition**

Milk Fat Composition

- Walloon Region of Belgium:
  - Collecting fatty acid composition since March 2005
  - First experimental on 25 farms
  - Currently nearly all cows under milk recording

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  - 864,835 test-days (all-lactation)

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- Next step:
  - Development of a genetic evaluation system for milk fat composition

Data and Model

- Previous research done has shown for milk fat composition traits (e.g., Soyeurt et al., 2008):
  - Genetic variation and
  - Medium to high hertitabilities
- Some modelling issues however:
  - Repeated records
  - Longitudinal traits
  - Highly correlated traits
    - With traditional traits (milk, fat, protein)
    - Among different fatty acids and fatty acid groups

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Use of historical test-day data

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Large number of relevant traits

Data and Model

- Selection of traditional traits
  - Based on INTERBULL traits
    - Milk, fat, and protein yield
- Selection of milk fat composition traits
  - Based on potential place in breeding goal
    - Milk pricing
      - Saturated fatty acid content (SAT) in milk (g/100g)
    - Potentially health related
      - Monounsaturated fatty acid content (MONO) in milk (g/100g)
  - Prediction from MIR spectral data
    - Latest prediction equations
      - Developed in RobustMilk 7FP project (Soyeurt et al., 2010)

Data

- **MIR predictions of FA in milk**
  - **(Soyeurt et al., 2010):**

Content in milk	R^2cv	SECV	RPD
Saturated FA	1.00	0.05	15.7
Monounsaturated FA	0.99	0.04	8.9

R^2cv = cross-validation coefficient of determination; SECV = standard error of cross-validation; RPD= the ratio of standard deviation of reference values to the standard error of cross-validation

Data and Model

➢ Only first lactation (01/1974 – 02/2010)

Trait*	N	Mean	SD
MILK (kg)	6,749,239	16.96	6.83
FAT (kg)	6,746,993	0.68	0.29
PROT (kg)	6,727,524	0.56	0.22
PFAT (%)	6,746,993	4.02	0.72
PPROT (%)	6,727,524	3.33	0.40
SAT (%)	220,397	2.79	0.49
MONO (%)	220,396	1.15	0.24

* FAT = fat yield, PROT = protein yield, PFAT = fat content, PPROT = protein content, SAT = saturated fatty acid content in milk and MONO = monounsaturated fatty acid content in milk

Data and Model

- Only first lactation (for the moment)

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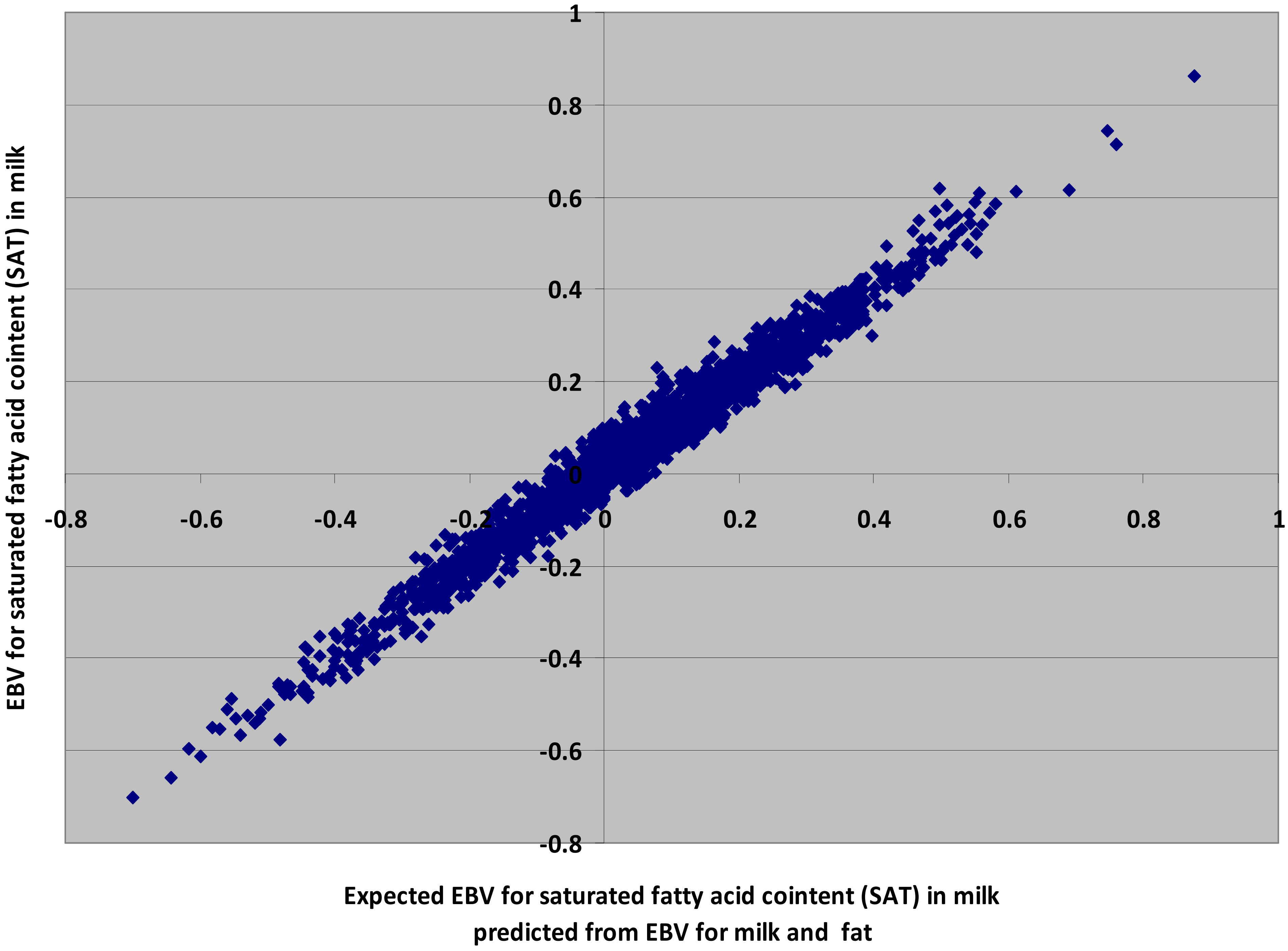
* MILK, FAT, PROT, SAT and MONO traits used in the evaluation

Expressing Results

- **Two potential components could contribute to selection objective**
  - **Milk pricing: SAT**
  - **Health related: MONO**
- **However underlying problem:**
  - **Both traits highly correlated to major traits**
- **Two consequences:**
  - **Risk of deleterious effects on current selection objectives**
  - **EBV of SAT and MONO expressing differences in MILK and FAT**



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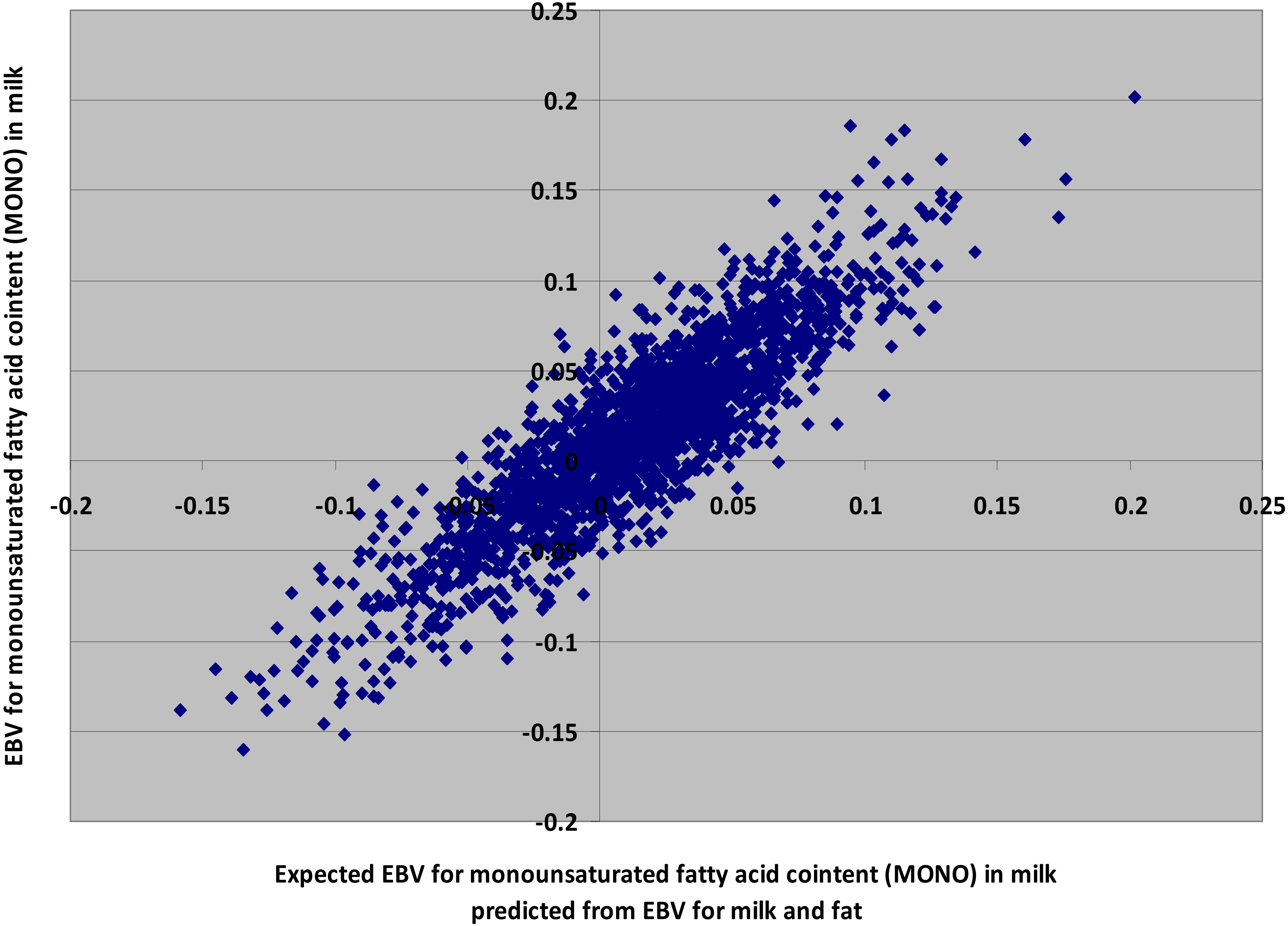
EBV for saturated fatty acid cointent (SAT) in milk
Expected EBV for saturated fatty acid cointent (SAT) in milk
predicted from EBV for milk and fat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EBV for monounsaturated fatty acid cointent (MONO) in milk
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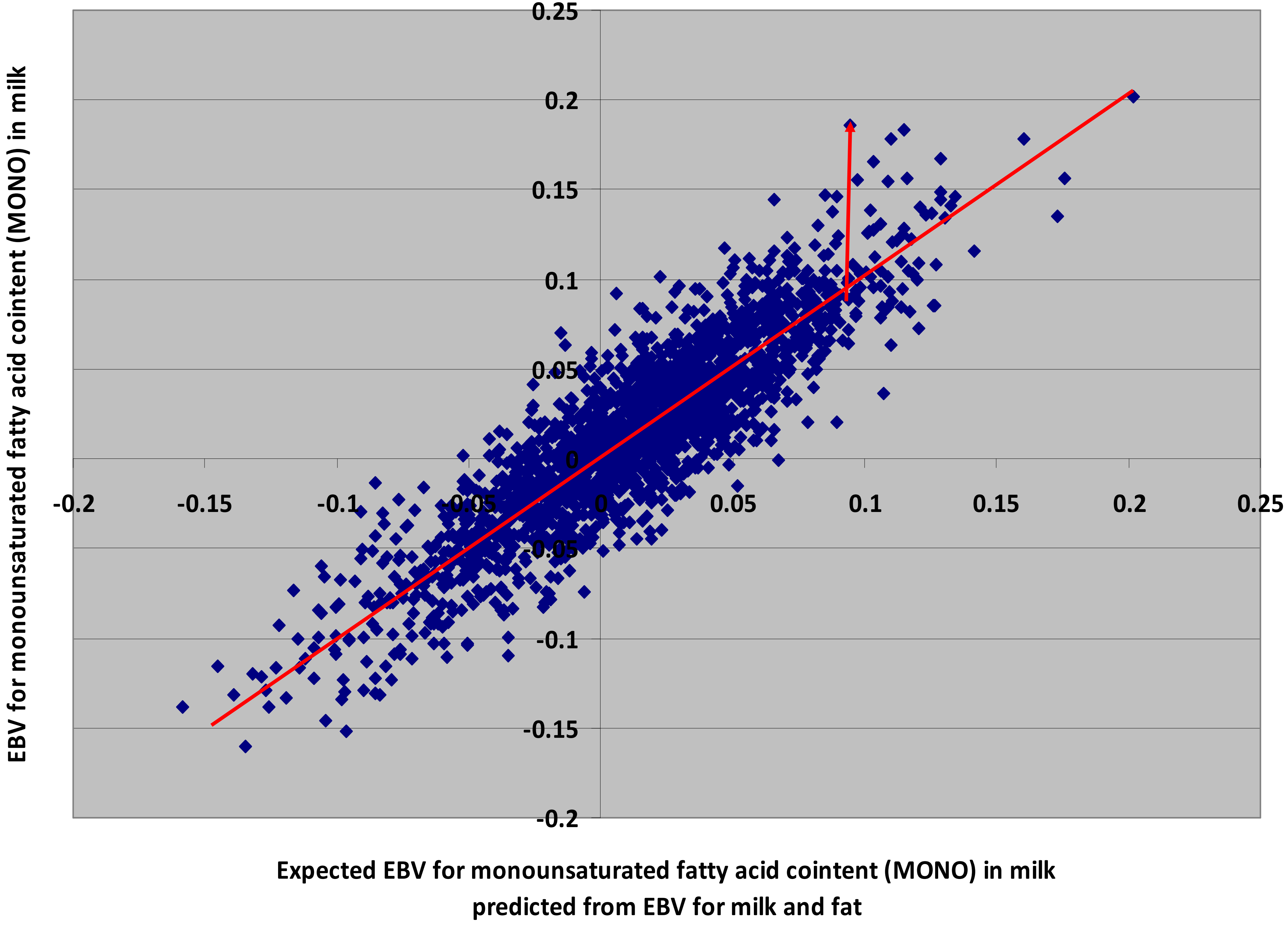
Expressing Results

- **Idea expressing relative differences**
- **Computation of new “traits” (indexes)**
  - **milk pricing: dUNSAT**
  - **health related: dMONO**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Expressing Results

- **Idea** expressing relative differences
- **Computation of new “traits” (indexes)**
  - **dMONO = MONO – E(MONO|MILK, FAT)**
  - **dUNSAT = – (SAT – E(SAT|MILK, FAT)**

Expressing unsaturation

Expressing Results

- **Idea expressing relative differences**
- **Computation of new “traits” (indexes)**
  - **dMONO = MONO – E(MONO|MILK, FAT)**
  - **dUNSAT = – (SAT – E(SAT|MILK, FAT)**
- **Altenative interpretation**
  - **dUNSAT and dMONO express in “fat”’**
- **Genetic parameters for dUNSAT and dMONO**
  - **Can be computed from (co)variance components**

Heritability

- **Heritability** of traits used for the 1st lactation

	h^2
Milk yield (kg/day)	0.31
Fat yield (kg/day)	0.33
Protein yield (kg/day)	0.25
Saturated FA (SAT) (%)	0.61
Monounsaturated FA (MONO) (%)	0.51
dUNSAT (%)	0.22
dMONO (%)	0.43

Correlations

- Genetic (above the diagonal) and phenotypic (below the diagonal) correlations among studied traits

	Milk	Fat	Protein	SAT	MONO	dUNSAT	dMONO
Milk yield		0.57	0.83	-0.42	-0.41	0.00	0.00
Fat yield	0.78		0.70	0.50	0.38	0.00	0.00
Protein yield	0.93	0.84		-0.11	-0.11	0.09	0.05
SAT	-0.32	0.34	-0.12		0.80	-0.11	-0.11
MONO	-0.33	0.23	-0.16	0.75		0.48	0.51
dUNSAT	-0.03	0.00	-0.01	-0.14	0.37		0.93
dMONO	0.00	0.05	0.01	-0.03	0.62	0.60	

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SAT	-0.32	0.34	-0.12		0.80	$\boxed{-0.11}$	$\boxed{-0.11}$
MONO	-0.33	0.23	-0.16	0.75		0.48	0.51
dUNSAT	-0.03	0.00	-0.01	-0.14	0.37		0.93
dMONO	0.00	0.05	0.01	-0.03	0.62	0.60	

The negative correlations confirmed that dUNSAT and dMONO represent the desaturation of fat (positive correlations with MONO)

Not more strongly negatively correlated because SAT is expressed in milk and not in fat

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Milk yield		0.57	0.83	-0.42	-0.41	0.00	0.00
Fat yield	0.78		0.70	0.50	0.38	0.00	0.00
Protein yield	0.93	0.84		-0.11	-0.11	0.09	0.05
SAT	-0.32	0.34	-0.12		0.80	-0.11	-0.11
MONO	-0.33	0.23	-0.16	0.75		0.48	0.51
dUNSAT	-0.03	0.00	-0.01	-0.14	0.37		$\boxed{0.93}$
dMONO	0.00	0.05	0.01	-0.03	0.62	$\boxed{0.60}$	

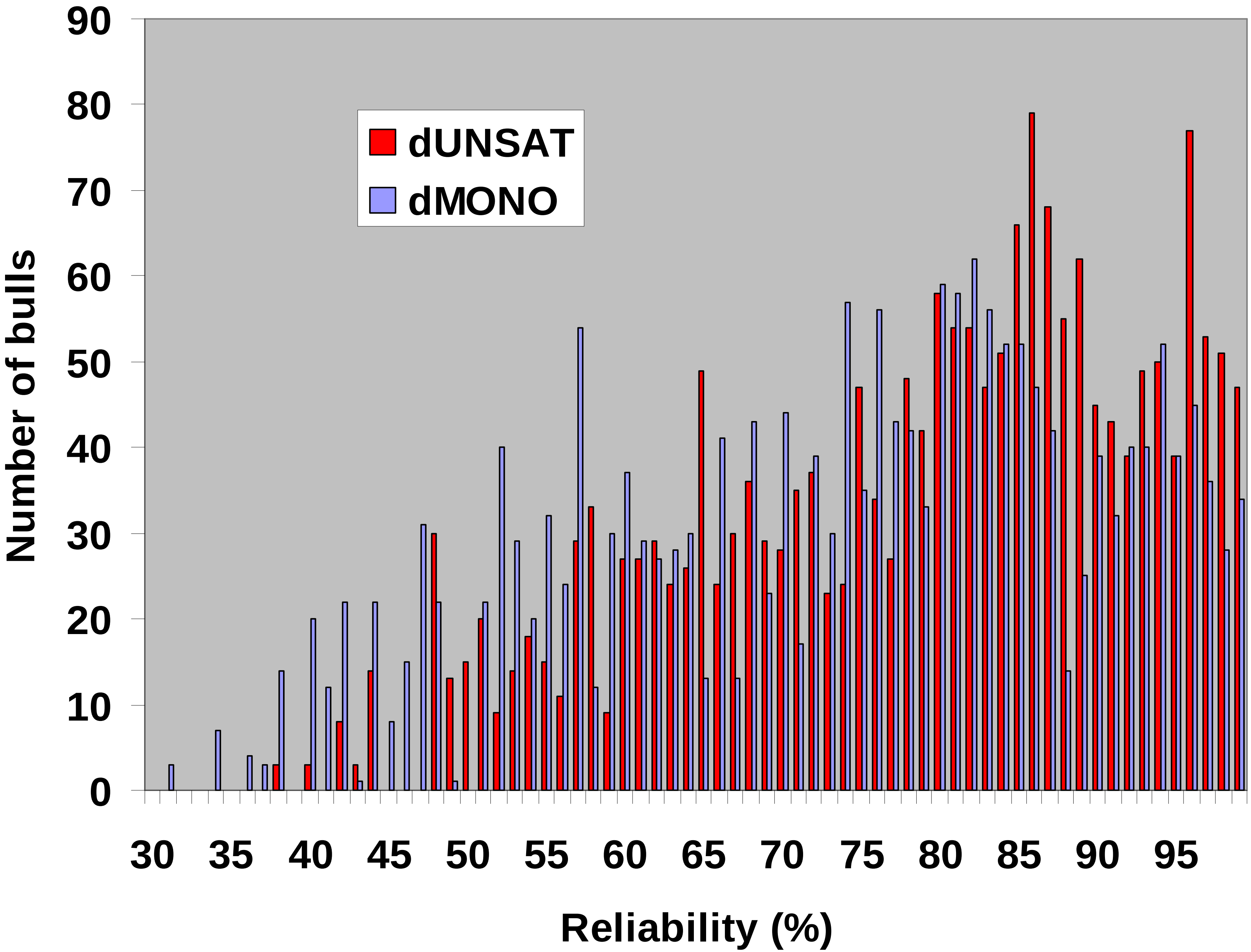
As expected dUNSAT and dMONO are positively correlated

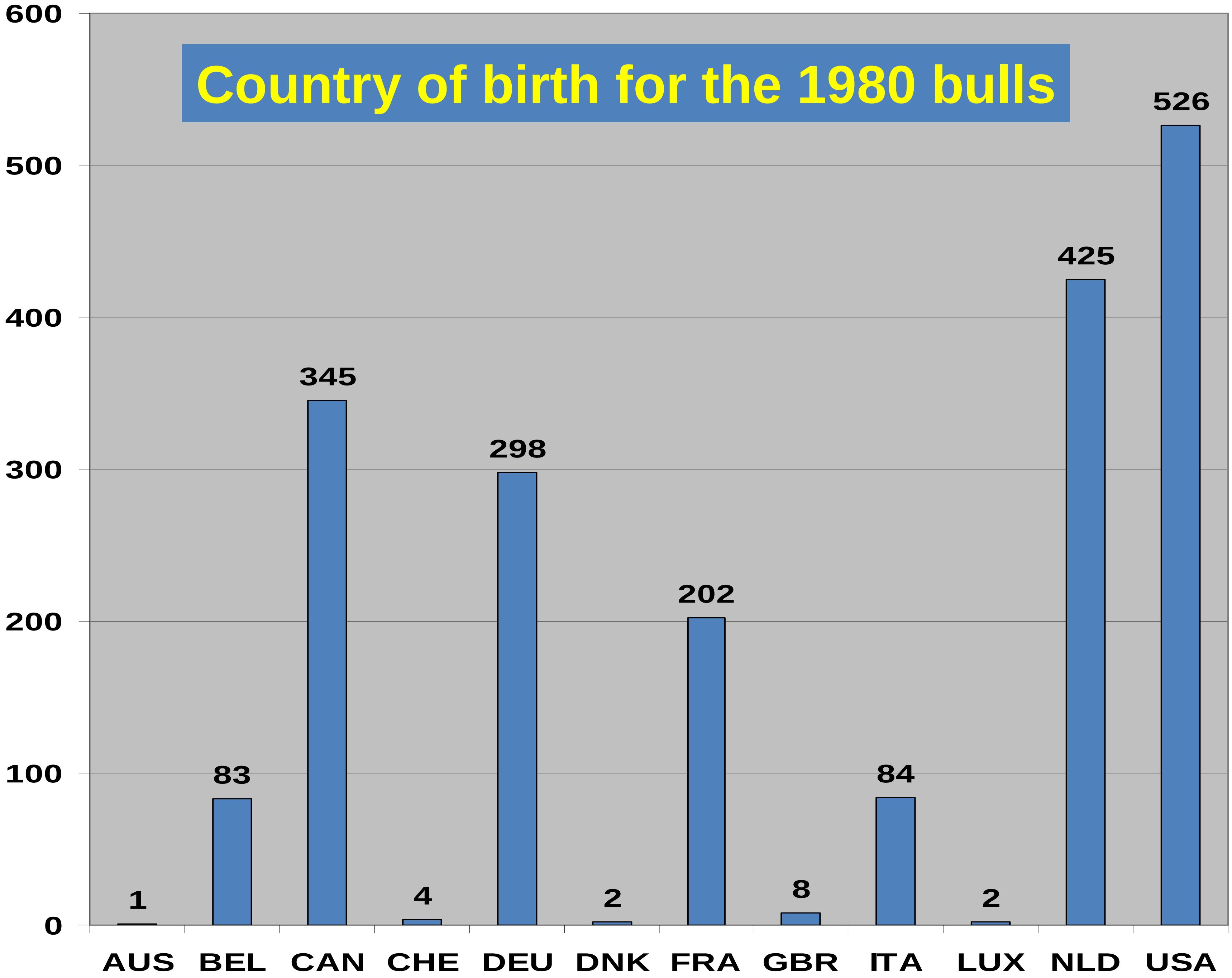
Not equal to 1 because dUNSAT takes also into account the contents of polyunsaturated FA

Results and Discussion

- **EBV and Reliabilities (REL)** for evaluated and expressed traits
- For 1980 bulls that had also MACE proofs

	EBV	REL	
Trait	SD	Mean	SD
Milk (kg)	485	0.75	0.16
FAT (kg)	16.7	0.79	0.14
PROT (kg)	13.5	0.74	0.16
SAT (%)	0.205	0.81	0.13
MONO (%)	0.052	0.68	0.19
dUNSAT (%)	0.035	0.78	0.14
dMONO (%)	0.024	0.74	0.16


dUNSAT
dMONO
Number of bulls
Reliability (%)
0
10
20
30
40
50
60
70
80
90
30
35
40
45
50
55
60
65
70
75
80
85
90
95


Country of birth for the 1980 bulls
600
500
400
300
200
100
0
1
83
345
4
298
2
202
8
84
2
425
526
AUS
BEL
CAN
CHE
DEU
DNK
FRA
GBR
ITA
LUX
NLD
USA

Results and Discussion

➢ **Correlation of EBV for milk composition traits with some traits and official indexes (very preliminary!!)**

	Trait*			Index*			
	SCS	LONG	FFERT	V€L	V€T	V€F	V€G
SAT	-0.04	-0.12	0.19	0.00	-0.15	-0.10	-0.08
MONO	-0.03	-0.08	0.14	0.08	-0.10	-0.08	0.01
dUNSAT	0.06	-0.11	0.03	0.04	0.01	-0.10	0.09
dMONO	0.05	0.07	-0.18	0.19	0.07	0.06	0.16

* Individual traits represent official EBVs computed during routine genetic evaluations or provided by INTERBULL. For more details please refer to http://www.elinfo.be.
FFERT = female fertility, SCS = somatic cell score, LONG = longevity.
V€L = subindex 'milk', V€T = subindex 'type', V€F = subindex 'functionality', V€G = global index

Conclusions

- First results **genetic evaluation system for milk fat composition** in Walloon Region of Belgium:
  - Still under development
  - Only current status
- Chosen traits showed:
  - **High heritabilities**
  - **Genetic variability**
- With still limited data:
  - **1906 sires**: EBV with REL ≥ 0.50 for **dUNSAT**
  - **1795 sires**: EBV with REL ≥ 0.50 for **dMONO**

Perspectives

- Adding more data:
  - Currently 500,000 records added every year
- Going to a multi-lactation model:
  - Better use of existing data from later lactations
- Adding new traits:
  - Additional fatty acids
- Integration of external information:
  - Different possibilities to be explored to integrate MACE EBV for MILK, FAT and PROT

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- And:

Genomic Prediction ????

Genomic Prediction

- Comparison to lists of gentyped bulls
  - Approximately 650 in lists and with REL ≥ 50% for dUNSAT and dMONO
  - Even if all bulls genotyped remains small population, very small for a reference population

- Some potential workarounds
  - Integration in BLUP of MACE proof of bulls for IB traits, would get EBV from correlated traits (and pedigree)
  - Maximizing REL of evaluated bulls by adding data (e.g., more lactations)
  - Adding reliable cows

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  - Maximizing REL of evaluated bulls by adding data (e.g., more lactations)
  - Adding reliable cows
- Best alternative single-step GBLUP?
  - Many advantages in this situation

gembloux agro bio tech

Université de Liège

A last take-home message:

Please stop throwing away your spectral data!

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Thank you for your attention!

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