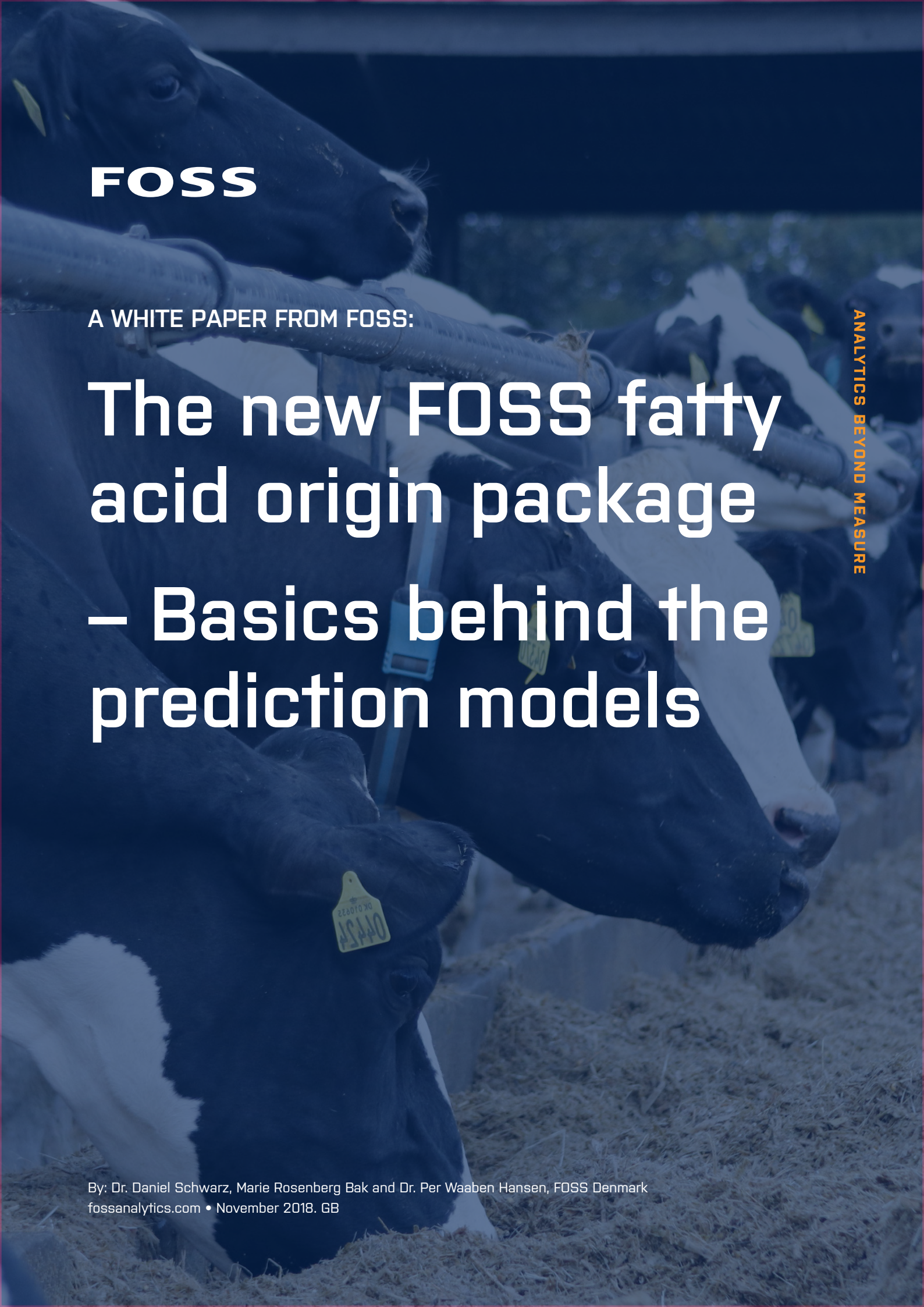

FOSS

A WHITE PAPER FROM FOSS:

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

ANALYTICS BEYOND MEASURE

By: Dr. Daniel Schwarz, Marie Rosenberg Bak and Dr. Per Waaben Hansen, FOSS Denmark

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Summary

Milk fatty acids are known to be closely related to the feeding of dairy cows and thus provide a lot of information on the nutritional status of dairy cows. Grouping the milk fatty acids according to their origin into *de novo*, mixed, and preformed fatty acids opens up the possibility to optimise management and, particularly, feeding of dairy cows. However, determination of fatty acids using FTIR (Fourier Transform Infra-Red) technology can be challenging due to the natural interrelation of total fat and the composition of fatty acids. We handled this natural interrelation by taking the total fat content of a sample into account and thus worked with the true composition of fatty acids. The performance of FOSS's new Fatty Acid Origin prediction models in terms of the correlation to the gas chromatography (GC) reference method for fatty acid analysis is very positive. The FOSS standardisation concept provides the basis for proper quality assurance. In the absence of fatty acid calibration samples that are fit for purpose, regular comparisons to GC results provide an alternative for assurance of reliability of fatty acid data generated by FTIR analysers.

Prediction Model Development

Background – Natural interrelation between fat and composition of fatty acids

The prediction of the composition of fatty acids using FTIR technology can be challenging given the natural interrelation between total fat and the composition of fatty acids (so called collinearity, see Eskildsen et al., 2014). In practical terms, this means that the higher the total fat content of a milk sample, the higher the content of specific fatty acids in that sample.

The above described interrelation must be considered during development of prediction models for fatty acids otherwise there is a danger that the model would predict fat rather than the composition of fatty acids as such. In this context, the composition of fatty acids can be determined using different units: 1) milk basis – g fatty acid per 100g of milk and 2) fat basis – g fatty acid per 100 g of total fatty acids. The fat content of a sample is taken into account using the 'fat basis' unit and thus the interrelation between total fat and fatty acids is considered. By design, the MilkoScan instrument determines fatty acids in the 'milk basis' unit, but results can automatically be converted to the 'fat basis' unit in Foss Integrator (i.e. calculated component) based on below equation (Figure 1).

$$\frac{g\ specific\ Fatty\ Acid\ /\ 100g\ Milk}{total\ Fat\% \cdot 0.95)} \cdot 100 = g\ specific\ Fatty\ Acid\ /\ 100g\ Total\ Fatty\ Acids$$

Figure 1. Equation for conversion between milk basis and fat basis (according to IDF Bulletin 447:2010)

The new FOSS Fatty Acid Origin Package

The new Fatty Acid Origin package consists of three individual prediction models being 1) *de novo* fatty acids, 2) mixed fatty acids, and 3) preformed fatty acids and is described in detail elsewhere (Schwarz, 2018). Briefly, fatty acids with 4-14 carbons are de novo synthesised in the mammary gland and thus considered as *de novo* fatty acids. Milk fatty acids with 18 or more carbons originate directly from the cows' diet and/or from lipolysis of adipose tissue (i.e. body fat mobilisation). The fatty acids C15:0 and C17:0 are known to be synthesised by the microbial flora of the rumen. Hence, C15:0, C17:0 and fatty acids with 18 or more carbons are considered preformed fatty acids. Fatty acids with 16 carbons can originate from *de novo* synthesis as well as the same sources as preformed fatty acids and are thus considered as mixed fatty acids (e.g. Jensen, 2002; Palmquist, 2006; Vlaemick et al., 2006).

Samples Used for Development of Prediction Models

The new FOSS prediction models were developed based on results of routine raw milk samples analysed in multiple countries on three continents (i.e. Europe, North America, Asia). Specifically, routine raw milk samples (both individual cow milk and bulk tank/pen samples) were selected based on total fat results as well as the fatty acid composition. All samples were selected using the 'fat basis' unit. In this way, the natural interrelation between fat and fatty acids was taken into account and it was ensured that the models would predict fatty acids as such, but not fat. All samples were analysed in replicate on MilkoScan instruments and afterwards by gas chromatography (GC).

The samples used for the development of the prediction models covered a wide range of total fat as well as fatty acid composition results (Table 1). The results were broader in range for individual cow milk samples than for bulk-tank milk samples. In general, the results covered the wide range of naturally occurring fat and fatty acid composition results.

In detail, samples were available from seven sites in USA, five in France, two in Canada, two in Japan, and one each in Denmark, Germany, the Netherlands, Norway, Spain, Sweden, and the UK.

Table 1. Total fat and fatty acid composition results of samples included in development of fatty acid origin prediction models. Fatty acid results are expressed in both units: milk and fat basis.

Fatty acid origin group									
Item	***De Novo***			**Mixed**			**Preformed**		
	n	Range	Mean ± SD	n	Range	Mean ± SD	n	Range	Mean ± SD
Total fat (%) – individual cow milk	388	0.84-7.27	3.88 ± 0.69	388	0.84-7.27	3.89 ± 0.69	388	0.84-7.27	3.89 ± 0.69
Total fat (%) – bulk tank milk	169	2.10-5.67	4.02 ± 0.51	169	2.10-5.67	4.02 ± 0.51	169	2.10-5.67	4.02 ± 0.51
All samples [g FA/100g milk]	557	0.21-1.70	0.95 ± 0.19	557	0.28-2.35	1.24 ± 0.28	557	0.29-3.25	1.35 ± 0.34
All samples [g FA/100g TFA]	557	16.5-33.7	25.5 ± 3.3	557	22.9-46.2	32.8 ± 4.7	557	21.9-61.3	36.2 ± 6.3
Individual cow milk [g FA/100g milk]	388	0.21-1.70	0.97 ± 0.21	388	0.28-2.35	1.24 ± 0.29	388	0.29-3.25	1.33 ± 0.37
Individual cow milk [g FA/100g TFA]	388	16.5-33.7	26.3 ± 3.5	388	24.0-44.2	33.5 ± 4.7	388	21.9-61.3	36.0 ± 7.0
Bulk tank milk [g FA/100g milk]	169	0.35-1.19	0.91 ± 0.12	169	0.73-2.07	1.20 ± 0.26	169	0.75-2.13	1.40 ± 0.27
Bulk tank milk [g FA/100g TFA]	169	17.1-28.1	28.1 ± 2.2	169	22.9-46.2	31.4 ± 4.3	169	24.3-48.0	36.6 ± 4.5

TFA = total fatty acids

The wide variation of results in the samples was desired and a result of the targeted selection of samples. Specifically, samples were selected to cover the full range of naturally occurring results, various different geographies (both across and within countries), various different cow breeds (e.g. Holstein-Friesian, Jersey, Ayrshire), different feeding regimes (e.g. total-mixed ration, pasture based feeding), and seasonal variation. This, in turn, was the basis for the development of prediction models that are robust towards a lot of variation (e.g. geography, breed, feeding). Moreover, only routine raw milk samples were included to ensure the best possible performance of the prediction models on routine samples afterwards (e.g. no risk of weakened performance by including artificially produced samples).

Method used for model development

The PLS (Partial Least Squares) regression method was used for development of the prediction models. This is a standard method applied for development of prediction models. Specifically, it is used to find the relation between the full spectrum data originating from MilkoScan instruments and the reference data from GC analysis. Given that the MilkoScan instrument determines fatty acids in the 'milk basis' unit, the models were developed based on this unit. To that end, all GC results, which come in the 'fat basis' unit, were converted to 'milk basis' using the MilkoScan fat percentage results (according to Figure 1).

Model Performance

The model performance was evaluated in two steps. In this context, all data available was used in a way that an independent validation of the developed prediction models was

possible. Specifically, in a first step, the actual PLS model was developed based on data from five sites in France and four sites in USA (total number of samples: 557, individual cow milk samples: 388; bulk tank/pen milk samples: 169). The developed models were then validated based on data from three sites in USA, two in Canada, and one each in Denmark, Germany, Japan, the Netherlands, Norway, Spain, Sweden, and the UK (total number of samples: 303, individual cow milk samples: 84; bulk tank/pen milk samples: 219).

The accuracy and repeatability of the new fatty acid prediction models are good (Tables 2 and 3). Accuracy was calculated based on the independent validation data, covering different sample types, instruments, seasons, breeds, and feeding programmes. The accuracy is expressed as $S_{x,y,}$ meaning the residual standard deviation/slope intercept corrected prediction error. Repeatability was calculated based on replicate analysis of samples on the MilkoScan.

Table 2. Accuracy, repeatability, and correlation coefficient of the new fatty acid origin prediction models, unit milk basis

Item	**Fatty acid origin group**		
	De novo	mixed	preformed
Accuracy, all samples	0.018-0.046	0.053-0.101	0.047-0.116
Accuracy, individual cow milk samples	0.018-0.046	0.053-0.08	0.047-0.116
Accuracy, bulk tank and pen samples	0.024-0.039	0.06-0.101	0.061-0.071
Repeatability, all samples	0.007-0.017	0.024-0.039	0.017-0.037
Repeatability, individual cow milk samples	0.007-0.014	0.024-0.039	0.017-0.028
Repeatability, bulk tank and pen samples	0.01-0.017	0.025-0.038	0.023-0.037
Correlation coefficient, R^2, all samples	0.93-0.99	0.92-0.97	0.82-0.99
Correlation coefficient, R^2, individual cow milk samples	0.95-0.99	0.93-0.97	0.85-0.99
Correlation coefficient, R^2, bulk tank and pen samples	0.93-0.99	0.92-0.94	0.82-0.97

Table 3. Accuracy and repeatability of the new fatty acid origin prediction models, unit fat basis

Item	Fatty acid origin group		
	De novo	mixed	preformed
Accuracy, all samples	0.47-1.25	1.40-2.82	1.12-2.71
Accuracy, individual cow milk samples	0.72-1.25	1.53-2.18	1.40-2.71
Accuracy, bulk tank and pen samples	0.66-1.02	1.48-2.82	1.41-1.89
Repeatability, all samples	0.21-0.45	0.62-1.10	0.55-0.99
Correlation coefficient, R^2, all samples	0.72-0.93	0.52-0.85	0.67-0.95

The validation confirmed good accuracy of the models because of a high correlation between results generated by MilkoScan instruments and gas chromatography (Table 2). Bulk tank and individual cow milk samples were evaluated separately, but the performance of the new prediction models was equally good on both types of samples (Table 2 and 3).

The relation between actual (i.e. GC) results and predicted (i.e. MilkoScan) results are further illustrated in Figure 2 (i.e. individual cow milk samples only) and Figure 3 (i.e. bulk tank milk samples only). In case of the three different models de novo, mixed, and preformed each, the data is closely positioned around the orange line, which represents ideal correlation between the two methods. This applies for both types of samples, individual cow milk as well as bulk tank/pen samples.

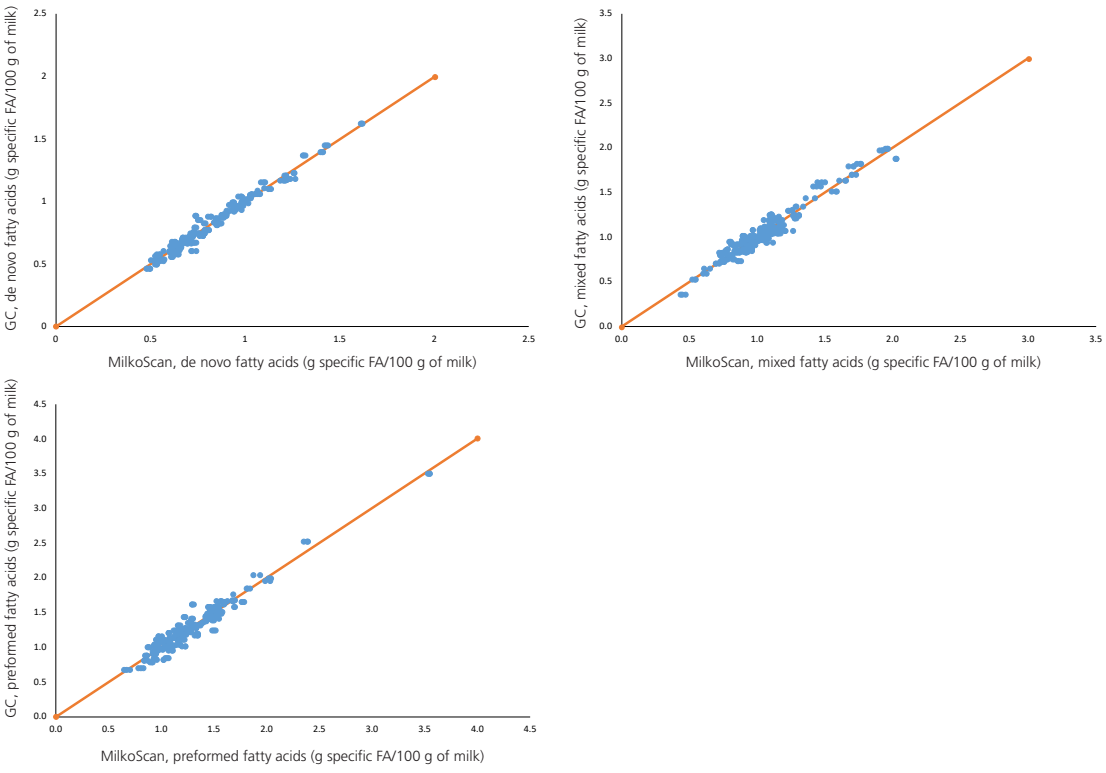

Figure 2. Relation between actual (GC) and predicted (MilkoScan) fatty acid results for a) de novo fatty acids, b) mixed fatty acids, c) preformed fatty acids based on results from 84 individual cow milk samples

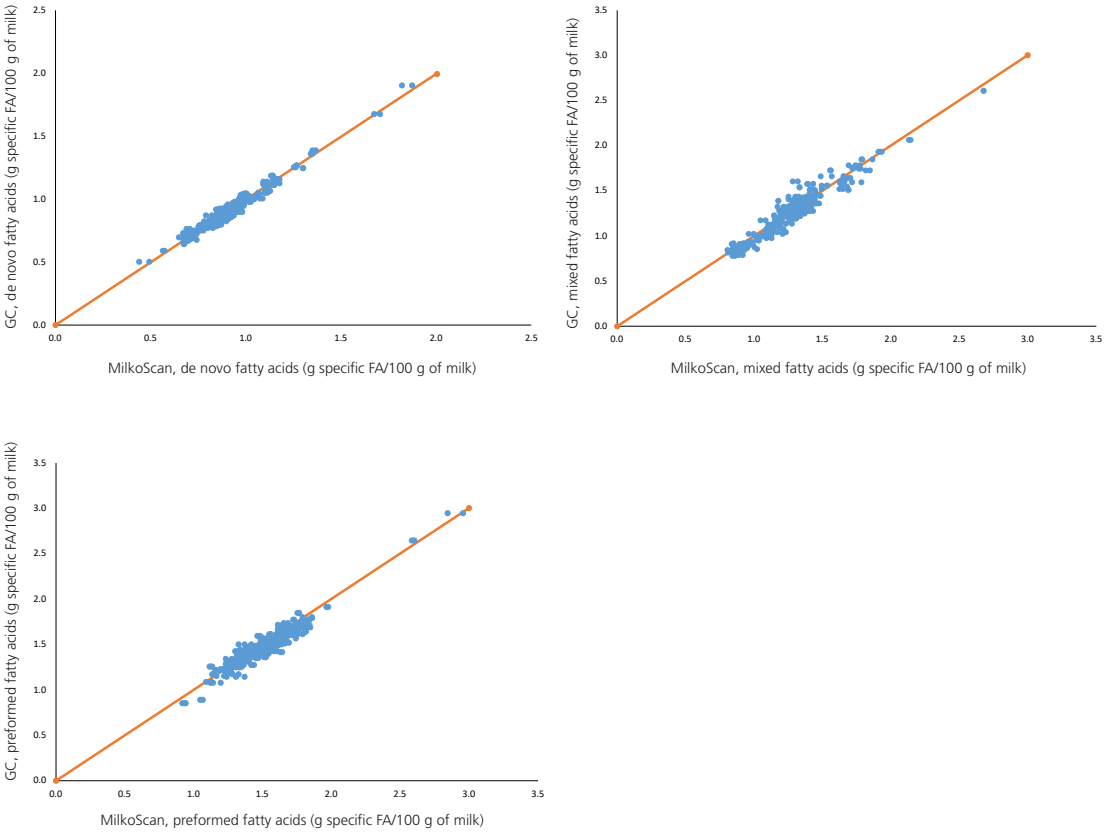

Figure 3. Relation between actual (GC) and predicted (MilkoScan) fatty acid results for a) de novo fatty acids, b) mixed fatty acids, c) preformed fatty acids based on results from 219 bulk tank/pen samples.

Quality Assurance

Quality assurance for fatty acid analysis consists of different elements. The first and most fundamental element is regular standardisation of the MilkoScan instrument, allowing transferability of prediction models as well as ensuring stability of the instrument over time as further explained below. More elements would be either the usage of calibration samples or comparison against the GC method for adjustment of prediction models as described further below.

The FOSS Standardisation Concept

FOSS has a unique standardisation concept as described in detail elsewhere (Winning, 2014). Briefly, this concept is applied on all FOSS FTIR analysers. The purpose is to ensure that instruments of the same type give the exact same results if measuring the exact same sample. This requires that the instruments generate the same spectral data, which in turn can be achieved through standardisation given that the FTIR Equalizer solution used for performing standardisation has a specific known spectrum the instruments adjust to during standardisation. Further, the FOSS standardisation concept provides the foundation for global prediction models which are transferable from one instrument to another instrument and even between different generations of instruments. Based on the FOSS standardisation procedure it is possible to correct for all potential differences between instruments' optics and compensate for instrument drift over time.

Calibration Samples

Calibration samples are used to harmonise results coming from different laboratories within certain geographical areas as well as to provide assurance on the reliability of results generated on FTIR analysers. The prediction models on FTIR analysers would typically be adjusted against the calibration samples. This is common practise for major components such as fat, protein and lactose.

In the case of fatty acid analysis, some institutions are offering calibration samples. These samples provide some variation based on the fatty acid origin groups or degree of unsaturation groups (Table 4). However, the results are expressed in the 'milk basis' unit and thus the actual total fat content of the samples is not taken into account.

Table 4. Example for fatty acid composition of a set of calibration samples. Fatty acids are expressed as in the milk basis unit (g fatty acid per 100 g of milk)

	Fatty acid origin groups			**Degree of saturation groups**			
Sample number	*De novo*	Mixed	Preformed	SFA	UFA	MUFA	PUFA
1	1.39	0.69	1.04	1.64	0.90	0.75	0.15
2	1.82	0.90	1.36	2.13	1.18	0.97	0.19
3	2.21	1.09	1.65	2.59	1.43	1.18	0.24
4	2.61	1.29	1.95	3.06	1.69	1.40	0.28
5	2.97	1.47	2.23	3.49	1.93	1.59	0.32

SFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = mono-unsaturated fatty acids, PUFA = poly-unsaturated fatty acids

Applying the unit fat basis for the same set of calibration samples shows that the true composition of fatty acids among the set of samples is exactly the same (Table 5). In other words, the set of calibration samples truly does not offer any variation. This is explainable by the natural interrelation of total fat and the fatty acid composition (Eskildsen et al., 2014). Moreover, the samples would be developed by adding different amounts of the same fat (and thus the same composition of fatty acids) to skimmed milk, which further highlights the lack of variation in terms of fatty acids.

Table 5. Example for total fat and fatty acid composition of a set of calibration samples. Fatty acids are expressed as in the fat basis unit (g fatty acid per 100 g of total fatty acids)

		Fatty acid origin groups			**Degree of saturation – groups**			
Sample number	Fat (%)	*De novo*	Mixed	Preformed	SFA	UFA	MUFA	PUFA
1	3.12	46.9	23.2	35.2	55.1	30.4	25.1	5.0
2	4.07	46.9	23.2	35.2	55.1	30.4	25.1	5.0
3	4.95	46.9	23.2	35.2	55.1	30.4	25.1	5.0
4	5.85	46.9	23.2	35.2	55.1	30.4	25.1	5.0
5	6.66	46.9	23.2	35.2	55.1	30.4	25.1	5.0

SFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = mono-unsaturated fatty acids, PUFA = poly-unsaturated fatty acids

Based on present knowledge, the above-described lack of variation in terms of fatty acid composition applies to any fatty acid calibration samples available on the market. Hence, as of today (November 2018) fatty acid calibration samples available on market are not fit for purpose. Actions have been initiated to develop calibration samples that offer the required variation allowing proper adjustment of FTIR instruments and thus harmonisation of results within the industry. In the meantime, adjustment of FTIR instruments against the GC method based on the results of routine samples offers an alternative to assure reliability of the fatty acid data generated.

Outlook and Support

The novelty of this type of fatty acid analysis requires a close collaboration between FOSS and different industry partners such as milk-testing laboratories and dairy cow nutritionists. Several projects were defined and are on-going. New developments, in particular in terms of the actual practical application of fatty acid origin results, will be communicated in dedicated seminars and at key industry events.

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FOSS

FOSS
Foss Allé 1
DK-3400 Hilleroed
Denmark

Tel.: +45 7010 3370
Fax: +45 7010 3371

info@foss.dk
www.fossanalytics.com