The new CombiFoss™ 7 DC – An update on differential Somatic Cell Count and other advancements in milk testing

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Summary

The new CombiFoss™ 7 DC is the latest generation milk analyser from FOSS. The instrument allows to test raw milk for up to 19 parameters, including the new Differential Somatic Cell Count (DSCC) parameter, simultaneously at a speed of up to 600 samples per hour. DSCC represents the proportion of specific immune cells (neutrophils (PMN) and lymphocytes vs. macrophages) and thus provides more information about the actual udder health status of dairy cows. This, in turn, opens up the possibility to develop new tools for improved management of mastitis, the most costly disease in dairy farming. Numerous activities validating the practical application of DSCC as a new supporting tool for mastitis management through DHI (dairy herd improvement) testing are ongoing. The main objective of this paper is to provide an update on the status and first outcomes of these activities.

In a first study, the development of the two parameters SCC and DSCC before, during, and after artificially induced mastitis under controlled conditions was investigated. Briefly, both SCC and DSCC increased evidently after the induction of mastitis. Interestingly, DSCC increased significantly even when the observed SCC increase was only moderate. The results of the study indicated that the combination of SCC and DSCC opens up the possibility to determine the stage of mastitis (i.e., early vs. late stage).

Another study aimed for the investigation of the development of SCC and DSCC in fresh lactating cows over a period of 10 days. While SCC revealed to vary evidently, the DSCC parameter was consistently high in infected cows. Hence, the combination of SCC and DSCC could be used for enhanced mastitis monitoring (e.g., low SCC but high DSCC results as an indicator for mastitis).

Ketosis, a metabolic disorder of high-yielding dairy cows, is a prevalent challenge on dairy farms nowadays. The possibility of offering ketosis screening in the frame of DHI testing is utilised successfully in many countries around the world. This service has been described as simple, practical, rapid, inexpensive, and valuable.

Up to 19 different parameters can be determined using the CombiFoss 7 DC. This, in turn, allows milk-testing laboratories to offer a variety of services to their clients. The new DSCC parameter provides more detailed information on the actual inflammatory status of a cow's udder. In this context, various practical applications for enhanced

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mastitis management based on DSCC are currently under validation. Based on the results of a first study, the combination of SCC and DSCC could be used to differentiate between the early and late stage of mastitis. However, further work is needed to mature this application. Other newer value-adding milk testing services such as ketosis screening and fatty acid analysis are successfully used in many countries around the world.

Keywords: DSCC, mastitis, ketosis.

Introduction

FOSS has launched the 7th generation of the CombiFoss milk analyser, which allows testing for up to 19 parameters including the new Differential Somatic Cell Count (DSCC), in 2016. DSCC indicates the combined proportion of polymorphonuclear neutrophils (PMN) and lymphocytes in percent and is described in detail (e.g., method, technology) elsewhere (Damm *et al.*, 2017; Schwarz, 2017a). The DSCC parameter provides more detailed information on the actual udder health status of dairy cows and thus opens up the possibility for enhancements in mastitis management. Mastitis, the inflammation of a cow's udder, is still causing tremendous losses of •32 billion to the dairy industry worldwide and thus the most costly disease in milk production (Seegers *et al.*, 2003).

Ketosis, a metabolic disorder in high yielding dairy cows, where energy demands exceed energy intake is another issue causing significant economic losses on dairy farms nowadays. The incidence of ketosis has been estimated to be 25-60% in dairy herds with costs of 260 Euro per case (Mc Art *et al.*, 2013, 2015; Mahrt *et al.*, 2015). The possibility of using DHI samples and FTIR technology for herd level screening with good values for sensitivity and specificity has been demonstrated (de Roos *et al.*, 2007; Denis-Robichaud *et al.*, 2014).

The main objective of this work is to provide an update on the status as well as first outcomes of the different activities regarding development of practical applications of DSCC. Beyond that, a short update on the latest developments in terms of ketosis screening and fatty acid analysis will be provided.

The concept and technology behind differential Somatic Cell Count

The concept and technology behind the DSCC parameter have been described previously (Damm *et al.*, 2017; Schwarz, 2017a). Briefly, the new Fossomatic™ 7 DC allows to measure 2 parameters, SCC and DSCC, simultaneously at a speed of up to 600 samples per hour. The key elements of the new milk analyser are a new chemistry, a new incubation unit, and a new measuring unit and were described in detail elsewhere (Schwarz, 2017a). DSCC represents the combined proportion of PMN and lymphocytes in percent (Damm *et al.*, 2017; Schwarz, 2017a). While DSCC is low in healthy mammary glands, DSCC increases evidently in mastitic milk. Overall, DSCC provides more detailed information on the actual inflammatory response of the mammary gland (Damm *et al.*, 2017; Schwarz, 2017a; Schwarz, 2018).

Various activities in terms of the development of the practical application of DSCC were initiated. While 2 studies were completed and first results are available most of the other activities are still in progress.

Update on differential SCC

Development of DSCC and SCC during controlled mastitis

A study where mastitis was induced under controlled conditions was performed (Wall *et al.*, 2018). Briefly, 8 healthy dairy cows were recruited and treated with cell wall components, either lipopolysaccharide (LPS) or lipoteichoic acid (LTA), to induce a defined inflammatory response. With LPS from *Escherichia (E.) coli,* a bacterium usually associated with acute, clinical mastitis and LTA from *Staphylococcus (S.) aureus,* a bacterium often associated with chronic mastitis, bacterial cell wall components representing two very common mastitis-causing pathogens (Sampimon *et al.*, 2009; Schwarz *et al.*, 2010) were chosen. Milk samples were collected at the following time points:

1. Baseline (i.e., days -3, -2, -1).
2. Right before treatment (i.e. day 0).
3. Five hours after treatment (i.e., day 0.2).
4. Early cure phase (i.e., days 1 and 2).
5. Late cure phase (i.e., days 3, 4, 5, 6, 7, and 14).

The SCC increased significantly at time point 3 compared to time points 1 and 2 in cows treated with LTA from *S. aureus* (Figure 1). DSCC increased significantly, too. Both SCC and DSCC returned to pre-infection levels at time point 5. The development of both parameters was very similar in case of LPS (*E. coli*) treatments. The control quarters showed a minor but not significant SCC and DSCC increase each after treatments. Interestingly, the DSCC parameter even increased evidently in cases where SCC only increased moderately (to levels of <200,000 cells/ml). The results of the study are described in detail elsewhere (Wall *et al.*, 2018).

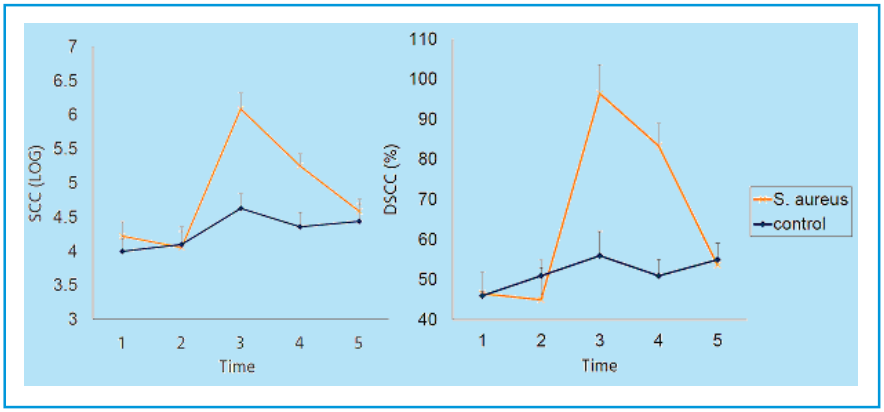

Figure 1. Development of SCC (logarithmic values) and DSCC in S. aureus treated (orange) and control (blue) quarter during the experiment. Time/time point:
1. Baseline (i.e., days -3, -2, -1);
2. right before treatment (i.e. day 0);
3. 5 h after treatment (i.e., day 0.2);
4. early cure phase (i.e., days 1 and 2);
5. late cure phase (i.e., days 3, 4, 5, 6, 7, and 14).

In summary, the study clearly showed that the SCC parameter increased significantly as a result of the induction of mastitis, as expected. DSCC increased significantly, too, thus clearly indicating the shift of cell populations from mainly macrophages to predominantly PMN. The results of the study support that the combination of SCC and DSCC opens up the possibility of determining the stage of mastitis. Specifically, high SCC (>200,000 cells/mL) and high DSCC values (>86%) could be used as an indicator for the early stage of mastitis. High SCC (>200,000 cells/mL) in combination with low DSCC values (<86%), however, could be an indication for the late stage of mastitis.

Development of DSCC and SCC over course of time

A study with the objective of investigating the development of DSCC and SCC in 20 fresh lactating dairy cows over course of time was performed. Cows were milked twice per day and milk samples (i.e., quarter foremilk and cow-composite) were collected at each milking for a period of 10 days (i.e., 18 sampling points). DSCC, SCC, and bacteriological analyses were carried out.

Healthy cows (i.e., no detection of mastitis pathogens) showed consistently low SCC (<50,000 cells/ml). DSCC values in samples with such low SCC are unreliable due to too low numbers of cells available for determination of DSCC (Damm *et al*., 2017). Cows infected with *S. aureus* indicated huge SCC variations, as expected (Harmon *et al*., 1994), whereas DSCC values were consistently high (exemplary: Figure 2).

In summary, the study showed that SCC varied quite evidently while DSCC results were rather at consistently high levels in infected cows. Thus, the combination of SCC and DSCC could be used for enhanced mastitis monitoring given that even cows showing unsuspiciously low SCC results would appear suspicious based on clearly elevated DSCC results.

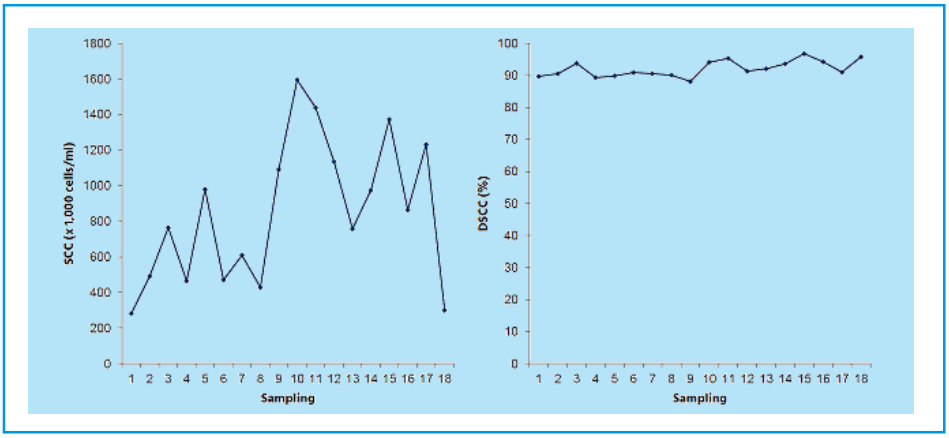

Figure 2. Example for the development of SCC and DSCC in cow-composite samples of one S. aureus-infected cow over a period of 10 days (2 milkings per day).

On-going DSCC projects

Numerous activities aiming for the development of DSCC applications in the frame of DHI testing are currently in progress. Specifically, two large-scale projects are running in Denmark and Germany. In the Danish project the data generation phase was just completed recently. 500+ dairy cows were tested (DSCC, SCC, bacteriology) once per month for a period of one year. In a next step, the data analysis will be performed. In the German project the Fossomatic 7 DC has been validated and implemented in routine operation. The next working package of the project is focused on the development of practical applications of DSCC.

Ketosis screening

Numerous applications for FOSS's ketosis screening calibrations were developed and are widely utilised by dairy farmers around the world, e.g. Ketodetect, CLASEL, France; Ketolab, Valacta, Canada; Ketomonitor, AgSource, US; Ketoscreen, CanWest DHI, Canada; ketosis screening by CRV and Qlip, the Netherlands, today. The service has been described as simple, practical, rapid, and inexpensive as well as highly valuable to milk recording clients as it elevates awareness of an otherwise undetected problem (Schwarz *et al.*, 2015). The service has actually helped to reduce the incidence of ketosis by 10% in Canada and France, as presented previously (Schwarz *et al.*, 2015). It was further seen that the keys to success in establishing ketosis screening as a service were the use of a quality assurance programme as well as proper and clear communication of test results to dairy farmers (Schwarz, 2017b).

Recently published articles describe milk BHB and effects of ketosis on the performance of dairy cows (Santschi *et al.*, 2016), risk factors associated with ketosis (Tatone *et al.*, 2017), and logistic and multiple linear regression models for prediction of ketosis (Chandler *et al.*, 2018) in detail.

Fatty acid analysis

Fatty acids can be categorised according to chain length and/or degree of saturation as well as the major fatty acids can be determined using FTIR technology. Fatty acid information are used for different purposes such as, e.g., optimisation of feeding of dairy cows (e.g., Visiolait application used in Germany and France) or production of value-added dairy products (i.e. products containing enhanced concentrations of unsaturated fatty acids – application in UK).

Conclusions

The CombiFoss 7 DC allows laboratories to measure up to 19 parameters in milk samples at low cost and at high accuracy, speed, reliability, repeatability, and robustness. This, in turn, allows milk-testing laboratories to offer a variety of services to their clients. Mastitis is still the most costly disease on dairy farms nowadays. The new DSCC parameter provides more detailed information on the actual inflammatory status of a cow's udder. In this context, various practical applications are currently under validation in several countries. Based on first results, the combination of SCC and DSCC could be used to differentiate between the early (high SCC, high DSCC) and late (high SCC, low DSCC) stage of mastitis. However, further work is needed to mature this application. Other newer value-adding milk testing services such as ketosis screening and fatty acid analysis are successfully used in many countries around the world.

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