The new CombiFoss 7 DC. Differential somatic cell count and other advancements in milk testing

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Abstract

FOSS has launched the 7th generation of CombiFoss milk analysers in October 2016. The new CombiFoss™ 7 DC seamlessly integrates MilkoScan™ 7 RM and Fossomatic™ 7 DC and allows to test raw milk for up to 19 parameters, including the brand new Differential Somatic Cell Count (DSCC) parameter, simultaneously in just 6 seconds. The objective of this work is to provide an overview on the key advances of the instrument and an update on the latest developments in terms of working with new parameters for milk testing, particularly DSCC, from around the world.

The MilkoScan™ 7 RM can be used to test for up to 17 different milk component parameters. The latest generation technology includes improvements of the optics and flow systems that result in better statistics, in particular for minor components such as urea and BHB (beta-hydroxybutyrate). Apart from that standardisation of spectra is still done using FTIR equalizer (FTIR - Fourier transform infrared spectroscopy), which is particularly important nowadays where full spectra information is utilised for various purposes.

The 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 module. Besides, the design of the instrument allows easy accessibility of the different modules inside the instrument.

DSCC is a new biomarker for mastitis management. Mastitis remains to be a significant challenge on dairy farms and still causes tremendous economic losses to the dairy industry. DSCC provides more information on the actual inflammatory status in the cow's udder by revealing the percentage of individual immune cells (i.e., DSCC represents the combined proportion of neutrophils and lymphocytes). Several research projects on the practical application of DSCC in the frame of dairy herd improvement (DHI) testing are currently running around the world.

A first research study, where the DSCC parameter was investigated before, during, and after artificially induced mastitis under controlled conditions was recently completed. The results showed that DSCC values changed significantly during the course of the experiment (i.e. <60%, >90%, and <70% before, during, and after infection, respectively). Hence, first indications on where to set a threshold for DSCC to distinguish between normal and active (e.g., mastitis) inflammatory response are available.

In conclusion, the new CombiFoss™ 7 DC allows highly accurate, fast, reliable, repeatable, and robust determination of up to 19 parameters from raw milk samples at low cost. DSCC is a new parameter providing more detailed information on the actual inflammatory response of the mammary gland and thus opens up the possibility to develop new tools for improved mastitis management that can be offered through DHI testing programmes.

Keywords: mastitis, ketosis, SCC, DSCC, dairy herd improvement testing, milk quality.

Introduction

The analysis of milk samples for payment and dairy herd improvement (DHI) purposes has evolved evidently since the 1970s where just milk fat, protein, lactose, and somatic cell count (SCC) were tested. Numerous new parameters were developed since serving optimisation of both milk quality and the management of dairy herds. Various new milk testing services based on new or newer parameters are currently in development are in the phase of implementation around the world. The overall aim of offering new parameters and milk testing services is to provide dairy farmers with more information for improved decision making.

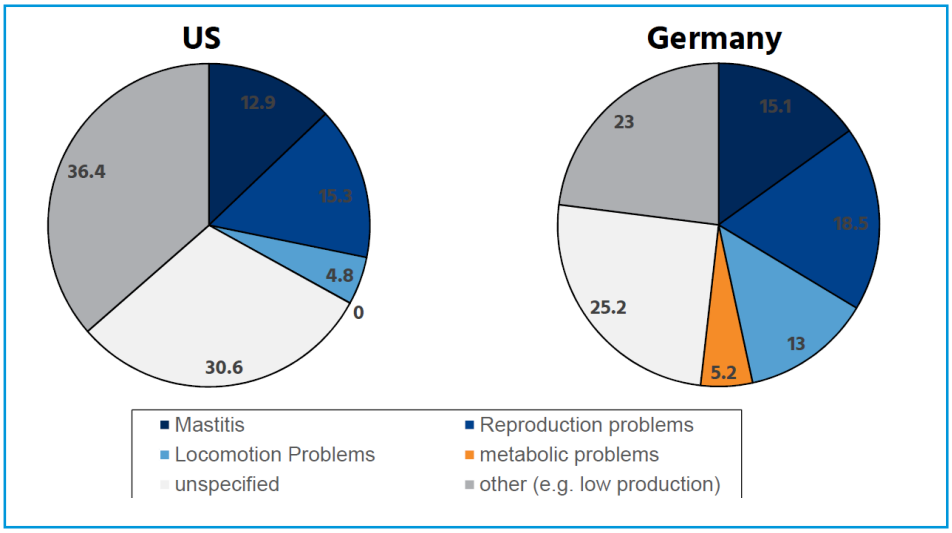

Figure 1. Overview on reasons for cows exiting dairy herds in the US (left) and Germany (right). Sources: CDCB, 2015; VIT 2016.

Mastitis, the inflammation of a cow's udder, is still causing tremendous losses of 32 billion Euros to the dairy industry worldwide (Seegers *et al.*, 2003). It is still one of the most common reason for cows to exit dairy herds in countries such as the US (9 million dairy cows) and Germany (4 million dairy cows) as illustrated in Figure 1. SCC is a well-accepted and broadly used indicator for mastitis and became a standard tool for mastitis management. However, SCC represents the total number of cells per ml of milk and does not harbour information about proportions of individual immune cells occurring in milk (i.e., macrophages, polymorphonuclear neutrophils (PMN), lymphocytes). Hence, a new parameter, Differential Somatic Cell Count (DSCC), indicating the combined proportion of PMN and lymphocytes in percent was recently developed (Damm *et al.*, 2017; Schwarz, 2017a). The percentage of macrophages is 100 - DSCC. It is well documented that both the total SCC and composition of the

immune cells change evidently during mastitis. Milk from healthy mammary glands is low in SCC that consist mainly of macrophages and lymphocytes (Lee *et al.*, 1980; Schwarz *et al.*, 2011a, b; Pilla *et al.*, 2012). However, SCC increases significantly and PMN are the predominant milk cell population in the presence of infection (Paape *et al.*, 2002). While SCC indicates the change in the total number of cells, DSCC reveals the change in the composition of the immune cells.

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 Euros per case (Mc Art *et al.*, 2013, 2015; Mahrt *et al.*, 2015). The possibility of using DHI milk 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).

FOSS has recently launch the 7th generation of the CombiFoss milk analyser, which allows testing for up to 19 parameters including the brand new DSCC. The objective of this work is to provide an overview on the key advances of the instrument and an update on the latest developments in terms of working with new parameters for milk testing, particularly DSCC, from around the world.

The new CombiFoss 7 DC

The new CombiFoss™ 7 DC seamlessly integrates MilkoScan™ 7 RM and Fossomatic™ 7 DC. It offers the possibility to test for up to 19 parameters, including the brand new DSCC parameter, simultaneously in just 6 seconds (at a speed of 600 samples per hour).

The MilkoScan™ 7 RM employs Fourier Transform InfraRed (FTIR) technology for measuring a full range of milk compositional parameters. Two types of material, diamond and calcium fluoride, of the cuvette are offered. Furthermore, the latest generation technology includes improvements of the optics and flow systems that result in better statistics, in particular for minor components such as urea and BHB (beta-hydroxybutyrate), due to an improved signal to noise ratio. Apart from that standardisation of spectra is still done using FTIR equalizer (FTIR - Fourier transform infrared spectroscopy), which is particularly important nowadays where full spectra information is utilised for various purposes.

The 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). Besides, the new modular design of the instrument allows easy accessibility of the different modules inside the instrument.

The new Differential Somatic Cell Count (DSCC) Parameter

DSCC is FOSS's new parameter for mastitis management. The concept and method behind the parameter have been described previously (Damm *et al.*, 2017; Schwarz, 2017a). DSCC represents the combined proportion of PMN and lymphocytes in percent and thus provides more detailed information on the actual inflammatory response of the mammary gland. This, in turn, opens up the possibility of developing new tools for improved mastitis management.

The development of the two parameters SCC and DSCC before, during, and after artificially induced mastitis under controlled conditions was recently studied in detail (Wall *et al.*, 2017). Briefly, both SCC and DSCC increased evidently after mastitis was induced. Interestingly, DSCC increased significantly even when the observed SCC increase was moderate only. SCC and DSCC returned to normal levels within a couple of days after mastitis had been artificially induced.

In general, the combination of SCC and DSCC allow a more detailed description of the udder health status of dairy cows compared to SCC alone. The International Dairy Federation (2013) recommended a SCC threshold of 200,000 cells/ml for differentiation between normal/healhty and (unspecific) mastitis. The novelty coming with the availability of DSCC information is the possibility of distinguishing between active or inactive inflammatory response as well (Figure 2). In this context, a threshold for DSCC might be at a level of 75%, however, it requires further research to determine that threshold precisely. The following 4 groups could be described working with SCC and DSCC:

- Low SCC and low DSCC: *normal/healthy*.
- Low SCC and high DSCC: *onset/early stage of mastitis*, elevated proportions of PMN have been described in udder quarters with SCC <100,000 cells/ml and were interpreted as early inflammatory reactions that must be triggered by bacteria (Schwarz *et al.*, 2011 a, b; Pilla *et al.*, 2012).
- High SCC and high DSCC: A condition where the cow's immune system actively *combats mastitis pathogens*.
- High SCC but low DSCC: A condition where fairly high proportions of macrophages instead of PMNs occur. Scientific literature suggests that this happens in *chronically-infected cows* (Leitner *et al.*, 2000).

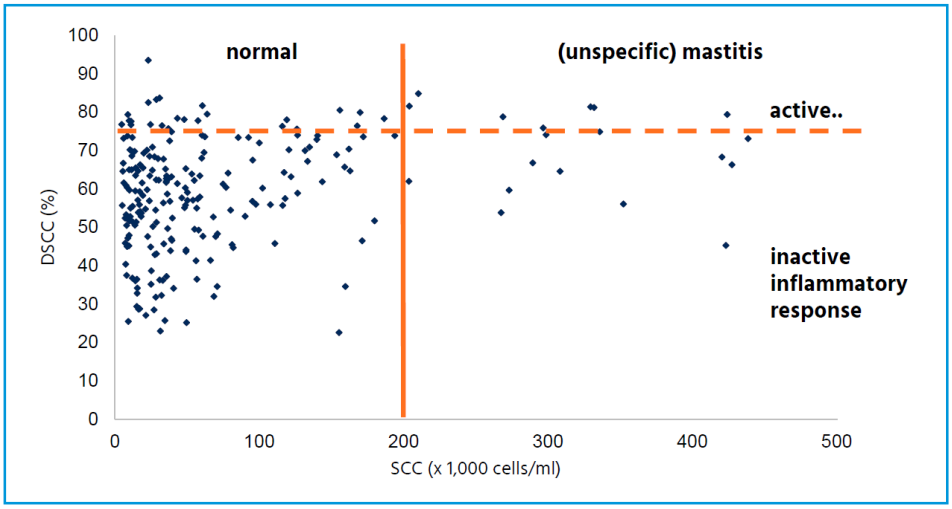

Figure 2. Example for SCC and DSCC results from a monthly DHI testing in a Danish dairy herd with 200 cows. Besides differentiation of normal and (unspecific) mastitis based on SCC, differentiation of active and inactive inflammatory response based on DSCC is possible. Each symbol represents the test result for one cow, overlapping is possible.

The 4 groups described and their interpretation is currently under investigation in various research projects around the world. Besides, specific applications for DSCC such as selective dry cow therapy, enhanced analysis of udder health in fresh lactating cows, and selection of milk samples for bacteriological testing (i.e., PCR) are currently under validation/in development.

Ketosis screening

Numerous applications for FOSS's ketosis screening calibrations were developed and are widely utilised by dairy farmers, 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).

Other applications

Two of the more recent other applications utilising FTIR spectra are adulteration screening and fatty acid profiling. Briefly, adulteration screening allows to screen for either intentional or unintentional adulteration of milk (e.g., in connection with payment testing) using targeted or untargeted models.

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 or production of value-added dairy products (i.e. products containing enhanced concentrations of unsaturated fatty acids).

Conclusions

The dairy industry demands new parameters that further help in terms of optimising milk quality and dairy herd management. The recently launched 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. Ketosis screening, which is well-accepted and valuable service in many countries, is one example for new, value-added services that can be offered to dairy farmers today. New services/ applications in terms of mastitis management based on FOSS's new DSCC parameter, which provides more detailed information on the actual inflammatory status of a cow's udder, are currently under validation in several countries.

List of References

CDCB (Council on Dairy Cattle Breeding) 2015. Reasons that cows in Dairy Herd Improvement programs exit the milking herd (2014). *https://www.uscdcb.com/publish/dhi/dhi16/cullall.html*

Damm, M., C. Holm, M. Blaabjerg, M. Bro Nielsen, & D. Schwarz. 2017. Differential somatic cell count - A novel method for routine mastitis screening in the frame of Dairy Herd Improvement testing programs. J. Dairy Sci. 100: 4926-4940.

de Roos, A.P., H.J. van den Bijgaart, J. Horlyk & G. de Jong. 2007. Screening for subclinical ketosis in dairy cattle by Fourier transform infrared spectrometry. J. Dairy Sci. 90: 1761-1766.

Denis-Robichaud, J., J. Dubuc, D. Lefebvre & L. DesCôteaux, 2014. Accuracy of milk ketone bodies from flow-injection analysis for the diagnosis of hyperketonemia in dairy cows. J. Dairy Sci. 97: 3364-3370.

IDF (International Dairy Federation). 2013. Guidelines for the use and interpretation of bovine milk somatic cell count. Bull. IDF 466/2013.

Lee, C.S., F.B.P. Wooding & P. Kemp. 1980. Identification properties and differential counts of cell populations using electron microscopy of dry cows secretions, colostrum and milk from normal cows. J. Dairy Res. 47: 39-50.

Leitner, G., E. Shoshani, O. Krifucks, M. Chaffer & A. Saran. 2000. Milk leucocyte population patterns in bovine udder infection of different aetiology. J. Vet. Med. B Infect. Dis. Vet. Public Health 47: 581-589.

Mahrt, A., O. Burfeind & W. Heuwieser. 2015. Evaluation of hyperketonemia risk period and screening protocols for early-lactation dairy cows. J. Dairy Sci. 98: 3110-3119.

McArt, J.A.A., Nydam, D.V. & G.R. Oetzel. 2013. Dry period and parturient predictors of early lactation hyperketonemia in dairy cattle. J. Dairy Sci. 96: 198-209.

McArt, J.A.A., D.V. Nydam & M.W. Overton. 2015. Hyperketonemia in early lactation dairy cattle: A deterministic estimate of component and total cost per case. J. Dairy Sci. 98: 2043-2054.

Paape, M. J., J. Mehrzad, X. Zhao, J. Detilleux & C. Burvenich. 2002. Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. J. Mammary Gland Biol. Neoplasia 7: 109-121.

Pilla, R., D. Schwarz, S. König & R. Piccinini. 2012. Microscopic differential cell counting to identify inflammatory reactions in dairy cow quarter milk samples. J. Dairy Sci. 95: 4410-4420.

Seegers, H., C. Fourichon & F. Beaudeau. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res. 34: 475-491.

Schwarz, D., U.S. Diesterbeck, S. König, K. Bruegemann, K. Schlez, M. Zschoeck, W. Wolter & C.-P. Czerny. 2011a. Flow cytometric differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J. Dairy Sci. 94: 5033-5044.

Schwarz, D., U. S. Diesterbeck, S. Koenig, K. Bruegemann, K. Schlez, M. Zschoeck, W. Wolter & C.-P. Czerny. 2011b. Microscopic differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J. Dairy Res. 78: 448-455.

Schwarz, D., D.M. Lefebvre, H. van den Bijgaart, J.-B. Daviere, R. van der Linde & S. Kold-Christensen. 2015. Global experience on ketosis screening by FTIR technology. ICAR Proceedings. ICAR Technical Series No. 19, ICAR, Rome, Italy, p 5-10.

Schwarz, D. 2017a. Differential somatic cell count - A new biomarker for mastitis screening. ICAR Proceedings. ICAR Technical Series No. 21, ICAR, Rome, Italy, p 105-112.

Schwarz, D. 2017b. Ketosis Screening in the Frame of DHI Testing - Usability and Experience from around the Globe. ICAR Proceedings. ICAR Technical Series No. 21, ICAR, Rome, Italy, p 93-99.

Wall, S. K., O. Wellnitz, R. Bruckmaier & D. Schwarz. 2017. Differential somatic cell count in milk before, during, and after artificially induced immune reactions of the mammary gland. J. Dairy Sci. (submitted)

VIT (Vereinigte Informationssysteme Tierhaltung). 2016. Annual report *http://www.vit.de/fileadmin/user_upload/wirsindvit/jahresberichte/vit-JB2016-gesamt.pdf*