Global experience on ketosis screening by FTIR technology

D. Schwarz[1], D.M. Lefebvre[2], H. van den Bijgaart[3], J.-B. Daviere[4], R. van der Linde[5] and S. Kold-Christensen[1]

[1]FOSS Analytical A/S, Foss Alle 1, 3400 Hilleroed, Denmark
das@foss.dk (Corresponding Author)
[2]Valacta, 555 Boul. des Anciens-Combattants, H9X 3R4, Ste-Anne-de-Bellevue, QC, Canada
[3]Qlip BV, Oostzeestraat 2a, 7202CM Zutphen, The Netherlands
[4]CLASEL, 141 Bd des Loges, 53940 Saint-Berthevin, France
[5]CRV BV, P.O. Box 454, 6800 AL Arnhem, The Netherlands

Abstract

The purpose of this study is to summarise the latest global experience on the application of a fairly new service that dairy herd improvement organisations can offer their customers - ketosis screening.

Ketosis is a metabolic disorder which usually occurs in dairy cattle during the early lactation period when energy demands for milk production exceed energy intake. This negative energy balance results in the cow using her body fat as an energy source, leading to an excessive accumulation of ketone bodies (i.e. acetone (Ac), β-hydroxybutyrate (BHB)) in blood as the fat is broken down faster than the liver can process it.

The Fourier Transform InfraRed (FTIR) spectrometry method developed for measuring ketone bodies in milk indicated adequate correlations with chemical method results and was proven to be valuable for screening dairy herds for the occurrence of ketosis.

In Quebec, Canada, Valacta has started offering the Ketolab ketosis screening service based on the measurement of BHB in milk as an option to farmers since April 2011. The service is optional and subject to an extra fee. Approximately 71% of farms have used the service in 2014 and over 54% of cows are now screened for ketosis. In 2011, prevalence of ketosis was 26% and has been declining steadily to 15% in 2014. Compared with negative (BHB <0.15 mM/l), cows with elevated (≥ 0.20 mM/l) BHB produced 2.4 kg less milk on test day, had higher fat and somatic cell count (SCC) and lower protein and urea content in milk. Reproductive performance was also severely affected by ketosis: cows with elevated BHB in early lactation had 24 more days open than negative cows. Furthermore, hyperketotic cows were 41% more likely to leave the herd before the next lactation.

In France, CLASEL offers the FOSS Ac and BHB calibration consolidated in a bio-model called CetoDetect® to predict the risk of ketosis based on the analysis of regular milk recording samples. The scale ranges from 0 to 5, where 0 = healthy animals; 1 and 2 = subclinical ketosis; 3 to 5 = clinical ketosis. Overall, the prevalence of ketosis varied between 10 and 30% according to the season and thus the feeding. Lower values were associated with high quality of grass for grazing during the spring months, whereas the prevalence increased when low quality silage was fed over the winter. Evidently higher values for milk yield and fertility parameters but lowest for SCC values were observed in cows with a score of 0 compared to those with a score of 5. The ketosis testing service is offered for 3 • per cow and year and utilised by approximately 50% of all farmers.

In the Netherlands and Flanders, Belgium, ketone bodies are routinely measured with milk recording analysis at Qlip. FTIR predictions for Ac and BHBA are combined with a few cow-related parameters into a binary (yes / no) score for ketosis developed by CRV. Ketosis scores for cows in the first 60 days of lactation are provided to dairy farmers through the milk recording report and are also used by the feed companies for evaluation of the transition period and the ration. The prevalence of ketosis in the Netherlands is 16%. Ketosis is a moderate heritable trait (heritability of 20%). Breeding values for ketosis are published since December 2014 and are part of the CRV breeding index "Better Life Health". This new service to provide routinely ketosis scores for fresh cows is well valued by the dairy farmers and feed companies.

In conclusion, screening for ketosis using milk Ac and BHB levels clearly indicates metabolic challenges in early lactation that have profound negative effects on subsequent performance. The service is profitable with a return on investment of about 10 to 1 and has also an added value in terms of breeding purposes. Ketosis screening offers high value to milk recording clients and elevates awareness of an otherwise undetected problem. This in turn can help reduce the incidence of the problem.

Keywords: ketosis, Fourier Transform InfraRed (FTIR), early lactating cows, screening tool.

Introduction

Ketosis or hyperketonemia is one of the most frequent metabolic disorders in high-producing dairy cattle, occurring typically in the first two month after calving. It is caused by a severe negative energy balance, where energy demands for milk production and body maintenance exceed energy intake. To compensate, cattle mobilise their body fat. Free fatty acids thus released from adipose tissue can then be used as an energy source or incorporated into milk fat. Hyperketonemia arises when the rate of release of fatty acids exceeds the ability of the liver to process intermediates of fatty acid oxidation, resulting in an accumulation of ketone bodies (i.e. acetone (Ac), β-hydroxybutyrate (BHB) and acetoacetate (AcAc)). Hyperketo-nemia negatively affects milk yield, reproductive performance, and increases the risk of subsequent diseases such as clinical ketosis, displaced abomasum, metritis, and lameness (e.g., Opsina *et al.*, 2010; Duffield *et al.*, 2009). The costs per case of ketosis has been estimated to be 289 US$ (McArt *et al.*, 2015).

In the absence of clinical signs in cases of subclinical ketosis (Andersson, 1988), diagnosis of this common type of hyperketonemia depends solely on the measurement of ketone body concentrations in blood, milk, or urine. The gold-standard test is measurement of the blood BHB concentration by laboratory methods (Oetzel, 2004). For on-farm testing, electronic hand-held blood BHB meters with high accuracy and time-related benefits have been evaluated (Iwersen *et al.*, 2009). However, one of the drawbacks of such hand-held blood BHB meters is the additional farm labour resources required to systematically test all animals at risk for hyperketonemia.

Implementing a ketosis surveillance programme using monthly available milk recording samples in the frame of Dairy Herd Improvement (DHI) offers a more practical and less labour-intensive approach. Fourier Transform InfraRed (FTIR) spectrometry (Hansen, 1999; de Roos *et al.*, 2007) is nowadays a common applied technique for analysis of milk recording samples on fat, protein, and lactose in milk and more recently other minor components such as urea, BHB, and Ac. For milk Ac and milk BHB, the correlation coefficients between the FTIR predictions and the results obtained with segmented flow analysis (Skalar, the Netherlands) were around 0.80 (de Roos *et al.*, 2007) for log-transformed values. Studies from Canada and the Netherlands showed that using milk BHB and Ac measured in regular DHI samples, ketosis could be detected with a good sensitivity (69 and 87%) and a very high specificity of 95% (de Roos *et al.*, 2007; Denis-Robichaud *et al.*, 2014). Hence, milk Ac and milk BHB are valuable parameters for screening herds on the occurrence of subclinical ketosis (de Roos *et al.*, 2007; Denis-Robichaud *et al.*, 2014)

The objective of this study is to compile an overview on the latest global experience on the application of ketosis screening as a new tool offered by DHI organisations.

Material and methods

Determination of milk BHB and acetone values

The observations are based on routinely performed DHI testing in Canada (region Quebec), Belgium (region Flanders), France (regions Pays de la Loire and Centre) and the Netherlands from January 1, 2012 to December 31, 2014.

Milkoscan FT+ (FTIR) instruments (FOSS Analytical A/S, Denmark) with a FOSS calibration for Ac and BHB measurements were used for analysis of regular DHI samples.

The protocol was established and validated according to FOSS recommendations. Briefly, 2,000 milk samples were analysed by a segmented flow analyser and FTIR to build the calibration. For further validation another set of 1,500 samples was analysed. All raw data was processed in collaboration with FOSS.

At Valacta, Canada, 100 samples submitted for routine analysis of BHB are randomly selected to be analysed for BHB by reference method (Skalar continuous flow analyser, Skalar, the Netherlands) every month to validate FTIR predictions against reference values. Furthermore, BHB content of routine pilot samples is analysed by reference method. This allows to monitor predictions on each instrument every 20 minutes.

At CLASEL, France, determination of Ac and BHB in milk was established in 2012 as described elsewhere (Johan and Davière, 2014). A monthly validation of the FTIR calibration against the Skalar chemistry reference method on the basis of 100 pilot samples is performed.

Qlip, the Netherlands and Flanders, Belgium, still applies the original basic calibration established in 2006 (de Roos *et al.*, 2007). No slope adjustment and no bias setting based on outcome of repeated tests of pilot milk with a more or less constant average concentration of Ac and BHB was performed.

Classification and application of results

The predicted milk BHB concentration is expressed as mM/l.

At Valacta, results are reported as risk for ketosis: milk BHB <0.15 mM/l = low risk; milk BHB ?0.15-<0.20 mM/l = medium risk; milk BHB ?0.2 mM/l = high risk.

At CLASEL, results from a decision tree including milk Ac and milk BHB are reported in a scale ranging from 0 to 5, where 0 = healthy animals; 1 and 2 = subclinical ketosis; 3 to 5 = clinical ketosis.

At Qlip and CRV, FTIR predictions for milk Ac and milk BHB are combined with the ratio between fat and protein percentage, parity of the cow and month of milk recording into a binary (yes/no) score for ketosis.

Results and discussion

Ketosis screening on herd level is widely used in Canada, France, Belgium and the Netherlands where Valacta, CLASEL, and Qlip operate (Table 1). Between 48% (Canada) and 85% (Belgium and the Netherlands) of farms are enrolled for the ketosis screening service, thus leading to 51 to 90% of cows that were screened for hyperketonemia. The proportion of farms enrolled for ketosis screening has increased during the observed period.

Valacta and CLASEL indicate the risk for ketosis (low, medium, high) in the DHI report provided to their customers enrolled for ketosis screening. CRV processes the data generated at Qlip and indicates ketosis 'yes' or 'no' for individual cows in the DHI report. The occurrence of high risk cows decreased slightly from 5 to 4% in Canada and France, respectively, between 2012 and 2014 (Figure 1). Medium risk cows occurred evidently less frequently in Canada (2012: 19%; 2014: 11%) and France (2012: 22%; 2014: 13%).

Table 1. Overview on the proportion of samples, farms and cows under ketosis screening from January 1, 2012 to December 31, 2014.

Laboratory	Total number of DHI samples analysed	Proportion of samples with milk BHB analysis (%)	Proportion of farms using ketosis screening (%)	Proportion of cows under ketosis screening (%)
Canada	7,600,000	54	71[1]	54
France	9,600,000	100[2]	48	51
BE and NL[4]	35,000,000	100[3]	85	90

[1] Proportion of farms that used the service for at least one test-day.
[2] Ac and BHB values were predicted for all samples, but reported back to farms enrolled for CetoDetect® only.
[3] All milk recording samples; however, just reported back for cows with days in milk<60.
[4] Belgium and The Netherlands.

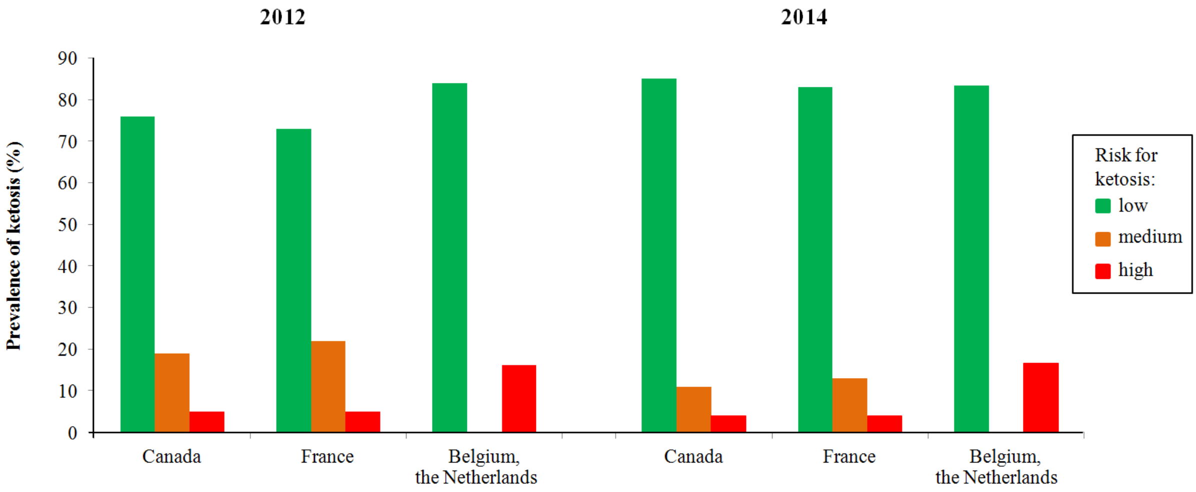

Figure 1. Prevalence of ketosis (low, medium, high risk) in Canada (Valacta), France (CLASEL) and Belgium (region Flanders) and the Netherlands (Qlip) in 2012 and 2014, respectively. Data for Belgium and the Netherlands are expressed as ketosis yes (high risk) or no (low risk).

The increased awareness and associated interventions due to ketosis screening might be an explanation for lower prevalences seen in 2014 (Figure 1). Nonetheless, experience from France shows that the quality of feed also contributed to this positive development. While the quality of corn silage harvested in 2011 was exceptionally poor, it was fairly high in 2013. The occurrence of cows classified as 'ketosis yes' in the Netherlands and Belgium, however, was steady at a level of 16.1% and 16.7% in 2012 and 2014, respectively (Figure 1). Ketosis screening was implemented in DHI programmes in the Netherland and Belgium already early 2011 and thus these countries have had a head start compared to Canada and France, where the service has been offered since late 2011 and early 2012, respectively.

The prevalence of ketosis varied among herds between 5 and 80%. It was generally higher in multiparous (26%) than in primiparous (20%) cows. The prevalence of ketosis further varied between 10 and 30% depending on the month of calving and thus the associated feeding during early lactation. Lower values could be found when cows were fed with high quality grass during the spring months compared to when low quality silage was fed over the winter.

Negative implications of ketosis in the early stage of the lactation on the subsequent performance of dairy cows were observed in all countries analysed. Cows with a high risk for ketosis produced between 2 and 6 kg and those with a medium risk between 1 to 3 kg less milk per day compared to the low risk group. Somatic cell count values were evidently high in the groups of high (>360,000 cells / ml) and medium (>318,000 cells / ml) risk cows (low risk group: 230,000 cells / ml). The risk of subsequent diseases such as clinical ketosis and displaced abomasums was also clearly increased in medium and high risk cows. Moreover, reproductive performance (e.g., calving to first service, non-return rate) of high and medium risk cows was clearly worse than of low risk cows. These observations are in accordance with the literature (e.g., Opsina *et al.*, 2010; Duffield *et al.*, 2009).

The presentation and interpretation of results from ketosis screening are exemplified based on experience from Valacta, where Ketolab is offered as a herd-level screening tool. Individual results are presented to farmers graphically in the form of a scatter graph of test-day BHB values by date of calving with an indication of the proportion of positive cows through time. In this way, trends over time can be visualized. A second scatter graph shows the current test day results by DIM at test-day, allowing to get a sense of when problems arise. A high frequency of positive cows in the first 2-3 weeks post partum points to metabolic issues related to nutritional status during the dry period (type II of ketosis, excess energy supply, over-conditioning, 'fat cow syndrome', insulin resistance, etc.) whereas hyper-ketonemia occurring further into lactation would relate to a large energy deficit in early lactation (type I of ketosis). Suggested corrective actions focus ration formulation, feeding management and environmental conditions (feed bunk access, overcrowding, cow comfort) during the relevant period. Individual results are also presented to farmers and they may elect to initiate preventative treatment on hyperketotic cows, but given the normal frequency of DHI testing and the window risk this is not the focus of the service.

At CRV, the information from ketosis screening is utilized for both animal health management and breeding. Lately, a breeding value for ketosis was introduced in the frame of the "Better Life Health" indicator. This value is available for all CRV bulls today and enables dairy farmers to breed for cows that are healthier. Hence, a decreasing prevalence of ketosis is expected in the long run.

Conclusions

The experience from 3 years of ketosis screening in Canada, France, Belgium and the Netherlands using FTIR technology on regular DHI milk samples clearly shows that this is a valuable service for farmers. Metabolic challenges of early lactating cows that have significant negative effects on their subsequent performance can be uncovered at low costs in a very practical way and subsequently addressed in a new way (i.e. critical evaluation of dry cow nutrition). The service is profitable with a return on investment of about 10 to 1 and has also an added value in terms of breeding purposes. Ketosis screening offers high value to milk recording clients and elevates awareness of an otherwise undetected problem. This in turn can help reduce the incidence of the problem.

List of references

Andersson, L., 1988. Subclinical ketosis in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 4: 233-248.

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.

Duffield, T.F., K.D. Lissemore, B.W. McBride & K.E. Leslie, 2009. Impact of hyper-ketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92: 571-580.

Hansen, P.W., 1999. Screening of dairy cows for ketosis by use of infrared spectroscopy and multivariate calibration. J. Dairy Sci. 82: 2005-2010.

Iwersen, M., U. Falkenberg, R. Voigtsberger, D. Forderung & W. Heuwieser, 2009. Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. J. Dairy Sci. 92: 2618-2624.

Johan, M. & J.B. Davière. 2014. Detection of ketosis in dairy cattle by determining infrared milk ketone bodies amount. 39th ICAR Session, Berlin, Germany, 19-23 May 2014.

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.

Oetzel, G.R., 2004. Monitoring and testing dairy herds for metabolic disease. Vet. Clin. North Am. Food Anim. Pract. 20: 651-674.

Ospina, P.A., D.V. Nydam, T.Stokol & T.R. Overton, 2010. Associations of elevated nonesterified fatty acids and ?-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93: 1596-1603.