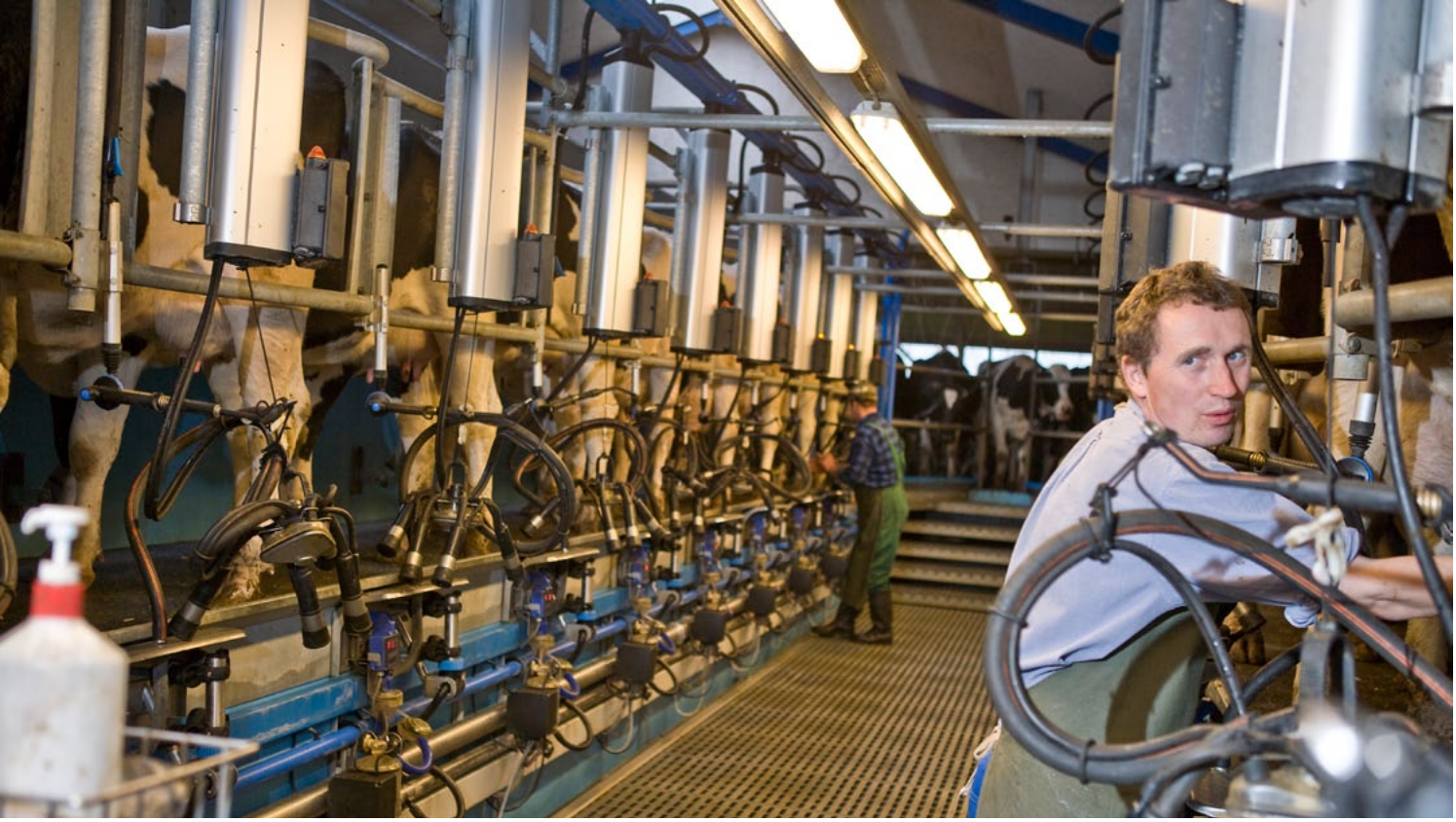

Status on Ketosis Screening

It is over a year since the launch of a new analytical tool which allows labs to screen milk recording samples for signs of ketosis as part of the conventional testing. Interest in the new parameter is growing, but there are questions too about how it should be implemented. Consultant Tove Asmussen gives an update on progress with the FTIR based screening tool and discusses the main considerations for new users.

Currently, four laboratories are using the screening tool. In Europe it is Qlip in the Netherlands, Analis in France and Milk Control Station Flandern in Belgium. In Canada it is Valacta, the lab covering the eastern part of Canada. The data now being sent to farmers is based solely on the FTIR results without reconfirmation from the direct method. This does not mean that laboratories are turning their back on the more precise direct method, but more of an acknowledgement of the FTIR screening as a faster and affordable 'indication' of the condition in the dairy herd.

Up and running in Europe and Canada

In Europe, MCC Flanders in Belgium and QLIP in the Netherlands started off with similar procedures. Until spring this year, both were only using results from the acetone prediction model. All results above a certain limit (in MCC Flanders 0.1 mmol/l and in Qlip the 4% highest results) were reconfirmed with the direct method before any results were forwarded to CRV*.

Since May 1st (MCC) and June 1st (QLIP) 2012 both labs have been forwarding results for both acetone and BHB to CRV. The labs no longer perform reconfirmation tests, saving both time and money. And, after evaluating large amounts of data, both labs have concluded that the new procedure provides results which are as good, if not even better than the previous procedure did.

In Canada, a study comparing results between the infrared method and the direct method revealed that on regular DHI samples, hardly any positive samples were detected to be high in acetone. This was probably due to

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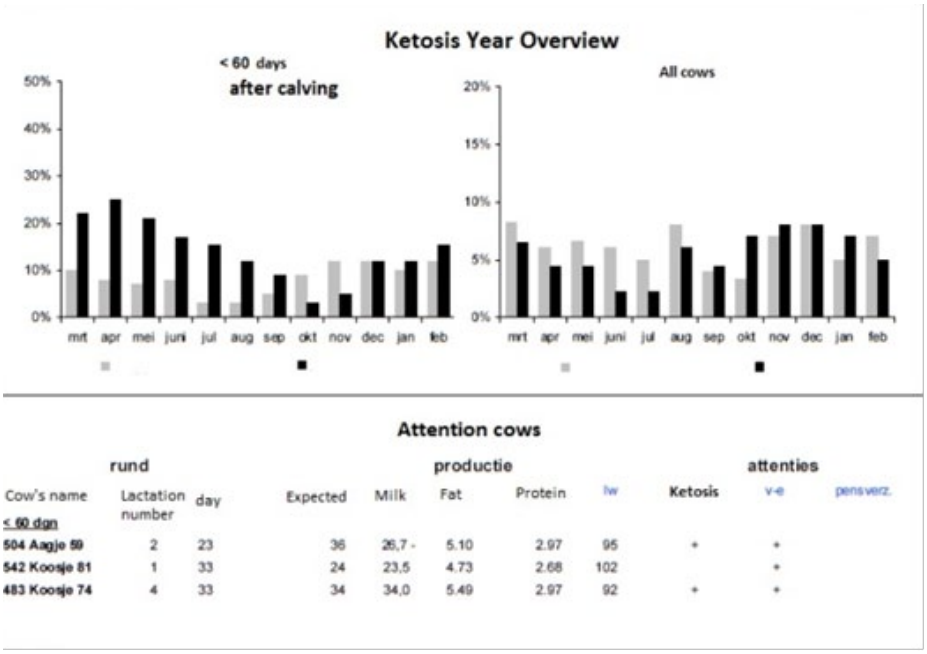

Example report showing the number of affected animals compared to same time last year for early lactation cows and all cows. Finally the specific cows affected are displayed also with information about lactation no and stage, actual yield compared to expected.

the sample handling procedure. Therefore it was decided to go for BHB alone.

Following evaluation of the prediction models, the direct method has only been used for monitoring performance of the prediction model. Before the routine screening started, advisors were invited to participate in education and discussions. Brochures were sent to all dairy farmers and the service was offered free, for three months. Today, approximately 65 % are requesting screening results and the number is increasing every month.

Things to consider in the upstart phase

There are a number of considerations when starting up, including how to communicate results, monitoring the performance of the prediction model, whether acetone or BHB should be selected and which preservatives can be used without affecting the results.

Communicating results

In Canada, results reported to dairy farmers show development in a herd over time - split up between 1st lactation and later lactation cows. Results for each cow are displayed month by month and listed within three groups; those being positive, above 0.2 mmol/liter, those being suspects and those below 0.15 mmol/liter.

This fits with existing practice for reporting in Europe where labs in Switzerland and France show the severity of the ketosis/acetone result for individual cows on a scale where level 1 indicates normal status and the higher the number, the more severe is the indication for ketosis.

In the Dutch and Canadian reporting, an additional overview of the situation and development of the entire herd is offered too. Given that ketosis is typically a herd problem, popping up periodically, this seems extremely appropriate for a diagnostic tool. Taking the precision into account it also seems appropriate to offer the result as an index between 1 and 5.

Reference analysis?

Performance can be verified by analyzing samples in parallel with a direct method onsite or by shipping the frozen, analyzed samples to a laboratory possessing the equipment. The samples must be analyzed on the Milko-Scan before they are frozen.

However, there are challenges verifying performance. As we are operating at a rather low level of concentration, it may be difficult to gather samples with high concentrations of acetone and BHB. So a more pragmatic method for adjustment of the prediction models may prove beneficial.

When analyzing large amounts of routine milk recording samples, most of these samples will contain no acetone at all and no or very low contents of BHB. Studies from the Netherlands and in Canada show that from 0 - 60 days after calving, a maximum of 30 % of all cows have elevated levels of acetone and BHB, and for entire lactation it is hard to imagine a percentage of elevated samples above 10%. This knowledge can be used to do a very pragmatic but, it seems, also very precise intercept adjustment of the prediction models.

Because we know the true value of the 50% lowest samples is 0 for acetone and approximately 0, - say 0.02 for BHB, the 50% fractile of the analyzed samples can be adjusted to zero for acetone and 0.02 for BHB. This is done by calculating the difference between 0/0.02 and the 50% fractile determined for each parameter. To check the 0-level for BHB from time to time and to

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check this procedure in general, it is recommended to take 50-100 random samples that have already been analyzed on a MilkoScan for analysis with the direct method at another laboratory.

Acetone or BHB?
Cows suffering from subclinical ketosis have, by definition, a concentration of acetone above 0.15 mmol/liter. For BHB the limit is 0.1 mmol/liter. In a study made at Qlip in the Netherlands back in 2005 it was found that prediction models for these two parameters could be made using FTIR.

Subsequently, we have seen that prediction of acetone in particular may be lower than expected, whereas the prediction of BHB is as expected on the same samples. This is probably due to the fact that acetone is more volatile than BHB.

Depending on local conditions, the prediction for BHB alone may detect most of the ketosis samples that would otherwise be detected in the lab. Thus it may seem natural to use the BHB prediction model only. However, samples high in acetone may not be high in BHB, so if the sample quality allows screening for acetone as well, this is the optimum solution.

Preservatives?
Studies indicate that the prediction model for acetone is not influenced by any of the preservatives in the test whereas the prediction model for BHB seems to be slightly influenced by sodium azide and to some extent by azidiol as well. If the concentration of these preservatives in the milk samples is constant, this can be compensated for.

Growing interest
For years, dairy farmers have known that ketosis can be a very costly disease and many have had a feeling that clinical cases were only the tip of the iceberg. In a Canadian study it was found that, on average, 30 % of early lactation cows in Canadian herds suffer from subclinical ketosis. Based on this paper and other publications, the losses due to subclinical ketosis are estimated by the Valacta laboratory to be 273 euro per case under Canadian conditions. Losses are due to lower milk production, more days open, increased risk of culling or death. With this knowledge in mind it is obvious that interest in integrating this parameter into routine milk recording services is increasing.

Three labs are now up and running, using FTIR, with well documented performance and good results. Many lessons have been learned along the way and now future users can start benefitting from all the experiences. A number of labs are already in the test phase and judging by the interest, others will soon join them.

By Tove Asmussen

The direct method
There is no universally accepted reference method for the direct (chemical) method referred to in this article, but this description taken from the Journal of Dairy Science Vol. 90 No. 4, 2007, gives an excellent summary of a commonly used chemical method.
"Chemical analyses were executed with segmented flow methods, applying SAN++ equipment (Skalar, Breda, the Netherlands). Acetone was determined through separation from the sample by gas diffusion through a Teflon membrane. The subsequent reaction with hydroxylamine resulted in a pH shift, which was measured photometrically at 520 nm using methyl orange as an indicator (Skalar method, catalog number 110-363).
Executed validation studies revealed a limit of detection of 0.06 mmol/L and a repeatability r of 0.06 mmol/L in the range up to 1.5 mmol/L. For the measurement of AcAc, the sample was preheated by placing a sealed container in a water bath at 100°C during 35 min. After mixing, the total amount of Ac and AcAc was determined according to the described procedure for the determination of Ac. The difference between the 2 values corresponded to the AcAc concentration.
The BHBA was determined by dialyzing a buffered milk sample against a Tris buffer solution (pH = 9). In the presence of 3-hydroxybutyrate dehydrogenase, BHBA was oxidized by NAD to AcAc and NADH.
The amount of NADH was measured photometrically at 340 nm (Skalar method, catalog number 388-301). The limit of detection was 0.04 mmol/L, whereas for the repeatability a value of 0.03 mmol/L was obtained in the range up to 0.6 mmol/L. Adding BHBA stock solutions to milk samples showed a recovery of 110%."