

Monitoring ketosis risk with milk MIR

MIR technology can be used to measure components in milk samples. One application is to monitor levels of ketones as an indicator of the risks of clinical and sub-clinical ketosis in cows in early lactation.

Australian researchers are evaluating the value of MIR as part of a regular herd test service. This fact sheet gives an overview of how dairy farmers and their advisors might use ketosis reports if they were included in herd test results.

Ketosis in dairy cows

Ketosis is a metabolic disorder in dairy cows. It usually occurs in early lactation when there is a huge increase in energy requirement for milk production and animals are unable to consume enough energy in their feed to meet their daily requirements. This is referred to as negative energy balance (NEB).

Negative energy balance is inevitable in cows around calving and persists for several weeks in early lactation. Freshly calved cows 'milk off their backs,' mobilising body tissue reserves and losing body condition until their daily feed intake increases to the point where energy intake meets their energy needs.

If cows experience excessive negative energy balance they may develop ketosis. This occurs when the liver cannot process all the excessive body fat that is being mobilised from body reserves, resulting in the production of ketones. The cow's ability to utilise ketones is limited. So cows in excessive negative energy balance or ketosis have raised levels of ketones circulating in their blood, urine and milk. An examples of these ketones is beta-hydroxy butyrate (BHBA).

Most dairy farmers are familiar with the signs of **clinical** ketosis: either lethargy and the typical sweet breath or excitability, poor co-ordination and possibly aggressive behaviour. They know it is a health crisis requiring immediate treatment.

Sub-clinical ketosis

However, **sub-clinical** ketosis has much greater impact on herd health productivity and profit because it affects many more cows in the herd.

Sub-clinical ketosis is referred to as a gateway disease as it can lead to other problems such as:

- Higher risk for abomasal displacement, retained foetal membranes, metritis, mastitis and lameness.
- Reduced fertility.
- Reduced milk yield.
- Increased likelihood of early culling.

Risk factors for sub-clinical ketosis include:

- Age (cows in second or later lactations at higher risk).
- Heavier body condition at calving.
- Increased colostrum yield at first milking.
- Season of the year (winter) and seasonal conditions.
- Prolonged length of dry period (and previous lactation length).
- Other health problems around calving.
- Male calf or dead calf.
- Poor calving ease breeding value.
- Elevated level of blood non-esterified fatty acid (NEFA) pre-calving (see below).

Although considerable research has been conducted overseas, there is very little information about the levels or costs of sub-clinical ketosis in pasture-based dairy production systems. NZ researchers (Compton and McDougall 2013) estimated the cost of sub-clinical ketosis at \$NZ86/case. A US study (McArt et al 2015) estimated \$US117/case allowing for the costs associated with reproduction, deaths, milk production, culling, drugs, labour and diagnostics. This figure went up to \$289 when the costs associated with displaced abomasum and metritis were included.

The extent and duration of negative energy balance in cows pre- and post-calving and the prevalence of sub-clinical ketosis varies widely between countries (see table),

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between herds and within herds from year to year. This makes it meaningless to generalise; investigations and advice must be specific for individual herds.

Table 1: Prevalence* of sub-clinical ketosis

Location	Study	Prevalence
Wisconsin, US	Oetzel, 2007 74 herds	16%
Canada	Geishauser et al 2001	40%
5 European countries	Berge et al, 2014	31 - 53%
Netherlands	Vanholder et al, 2015	47%
Denmark	Sloth et al, 2010. 70,000 cows	20%
UK	Macrae et al, 2012. 1,200 herds	27%
New Zealand	Compton et al, 2015 (overall incidence in first 5 weeks of lactation varied between herds 12-100%)	26-34%
New Zealand	Phyn, 2016 (overall incidence in first 5 weeks of lactation varied between herds 62-82%)	25 - 60% Peak prevalence in first 5 weeks lactation)
Australia	None to date	??

* Results are not directly comparable as different methods were used. However they demonstrate that sub-clinical ketosis is a common problem in dairy herds throughout the world.

Preventing sub-clinical ketosis

The use of an effective transition feeding program from 3-4 weeks before calving to four weeks after calving can help cows regain their appetite more quickly in early lactation and achieve higher feed intakes which reduces the risk of sub-clinical ketosis. The key principles are:

- Control body condition at calving so few cows are more than score 5.5.
- Maintain adequate availability and density of feed.
- Use more digestible forages with lower slowly-digestible fibre content.
- Don't feed too much rapidly-fermenting starch e.g. wheat.
- Provide access to feed for > 8 hours/day.
- Provide adequate feed access for all cows.
- Control dominance behaviour.

Treating sub-clinical ketosis

Cows with sub-clinical ketosis can be treated with 300ml propylene glycol by oral drench, daily for 3 to 5 days. However, the challenge is to be able to identify cows or herds at risk so that early intervention can be implemented. Milk MIR is a potential tool for early identification.

Monitoring ketosis risk

Currently there is no easy way to directly measure severe negative energy balance (ketosis risk) in grazing animals. Energy balance is the difference between energy intake and energy used and these are both impossible to routinely measure in a commercial dairy herd.

Energy balance can be indirectly monitored by measuring indicators in blood, urine or milk samples. All existing methods have limitations and none are used routinely on Australian dairy farms.

It would be desirable to monitor cows before calving to allow for early intervention. The pre-calving test is for blood levels of non-esterified fatty acids (NEFA). Samples need to be sent to a lab (there's no test strip kit) so there's a delay in getting the results. Timing can also be tricky as it's difficult to know exactly when a cow is going to calve.

In cows that have calved, measuring the level of the ketone, beta-hydroxy butyrate (BHBA), in blood is considered the gold standard test. Hand held meters (diabetic test kits) can be used on farm to provide an instant result. These work by measuring the concentration of ketones in a sample of blood placed onto the test strip and inserted into the machine. However, collecting blood samples from an entire herd on-farm presents many challenges for farmers or their vets, including costs and logistical complexity.

If blood BHBA testing is done on commercial herds, it usually involves sampling a small number of cows in the herd. This is usually only done on the advice of a vet, after a case of clinical ketosis.

NZ research

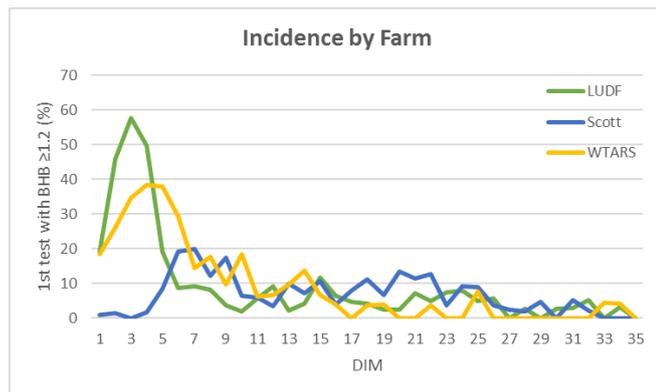
In 2016, DairyNZ conducted a large-scale ketosis study involving about 1000 cows across three herds.

The study confirmed that the use of the cow-side hand-held meters for monitoring blood BHBA of all cows in a herd was not feasible on commercial dairy farms. It was too logistically challenging and the cost of extra technical staff would make the exercise prohibitive. MIR has the potential to screen all cows in a herd for indicators of energy balance and ketosis from milk samples.

In the NZ study herds, the overall prevalence of sub-clinical ketosis during the first five weeks of lactation was 75% which is much higher than levels found in many international studies. The study also found significant variation between the herds in terms of:

- Peak prevalence during the first 5 weeks of lactation (25-60%) and timing of peak incidence of the first positive test for sub-clinical ketosis (3-7 days in milk and overall incidence (62%-82%) of sub-clinical ketosis.
- Its impact on production and fertility.
- The outcomes of interventions.

This variation is consistent with findings from research around the world and reinforces the need for monitoring on individual farms and herd-specific interventions.



Milk MIR to monitor ketosis

MIR technology offers the potential to screen milk samples for indicators of energy balance and ketosis risk. Milk MIR results could be used to provide alerts for early intervention, either at the individual cow or whole herd level.

If the analysis was done with milk samples from routine herd testing, all cows in the herd (rather than a small sample) could be tested with minimal extra effort or cost. This is a major advantage over blood tests for BHBA. However, it would probably require cows in early lactation to be herd tested more frequently than is the current practice.

MIRforProfit progress update

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MIR technology involves shining a light beam through a milk sample to measure the quantity of various milk components. It is used commercially to measure milk components such as fat %, protein % and fatty acids.

It can be used to measure other milk components that could be useful to dairy farmers. The MIRforProfit team is investigating two potential applications:

- for monitoring animal/herd performance of issues such as energy balance/ketosis risk and methane emissions.

- for developing Australian Breeding Values (ABVs) for new traits such as energy balance or methane emissions.

The MIRforProfit team has tapped into data, research findings and expertise from overseas. However, Australian research is needed to ensure MIR results are consistent and accurate for local conditions.

The MIRforProfit research involves two stages:

1. MIR prediction

- a. Developing equations for energy balance, milk fatty acids and methane emissions from milk MIR spectra (using cows from the Ellinbank herd which was intensively herd tested and genotyped).
- b. Refining the energy balance equations using data from commercial herds with detailed records, including fortnightly herd testing over spring. (Milk fatty acid and methane emission can't be validated in commercial herds due to the costs of collecting this data).

2. MIR validation

- a. Determining how closely the actual performance data matches the performance predicted from milk MIR results. (drawing upon data from 10,000 cows in commercial herds).
- b. Combining performance data with genotypes to develop an ABV(g) that reflects an animal's genetic potential for the trait (eg ketosis resistance).

MIR data for energy balance, ketosis risk

The application of MIR technology for the dairy industry is in early days. To create useful reports, MIR spectral data from milk samples need to be matched to cows with detailed performance records (phenotypes) for the trait of interest.

There are at least three options for matching energy balance data to milk MIR spectra:

1. Direct, using detailed records of energy intake and energy use from research herds.
2. Fatty acids in milk.
3. Ketones in milk or blood.

Energy intake and use

The Ellinbank research herd has facilities for measuring energy intake and use by dairy cows. The MIRforProfit and Ellinbank teams collected detailed data on energy balance and milk fatty acid composition which was matched to MIR spectra from milk samples from 120 cows in the Ellinbank herd during Spring 2016.

The accuracy of predicting energy balance using milk MIR from Australian cows was 0.4 which is similar to the prediction accuracy achieved by applying the UK equation

to Australian data. However, a higher accuracy is desirable and further work is needed to determine if it's possible to achieve the level of accuracy needed for commercial implementation of milk MIR predictions of energy balance.

Fatty acids in milk

Animals in negative energy balance have elevated levels of certain fatty acids in milk; for example, some C18 fatty acids are commonly associated with body tissue mobilisation (caused by negative energy balance).

Analysis of the results from cows in the Ellinbank herd, showed that the fatty acid C18:0 (stearic acid) has the strongest correlation to negative energy balance in Australian dairy cows.

Results from the Ellinbank study were used to develop equations to predict milk fatty acid profiles from milk MIR spectra. These equations were applied to data from about 2300 cows in five commercial dairy herds with excellent records.

The accuracy of MIR predictions for fatty acid are reasonably good, at > 0.9.

Ketones in milk or blood

While blood ketone levels are recognised as being a very reliable indication of sub-clinical ketosis risk, their use is limited by the logistical challenges of collecting blood samples in commercial herds.

MIR technology offers the potential to monitor ketone levels from milk samples, if an acceptable level of accuracy can be achieved.

In the UK, equations have been developed to match MIR spectral data to ketones in milk. DairyBio PhD student Tim Luke is collecting Australian data to develop equations to link milk MIR spectra to blood ketone levels in cows in early lactation. This work is scheduled for 2017-2020. This work should provide the data to develop the equations to predict ketosis risk from milk MIR results.

MIRforProfit early results Table 2: Prediction accuracy to date	
Trait	Accuracy
Energy Balance (validated in commercial herds –)	0.6
Milk fatty acid (C18) (validated in Ellinbank herd)	0.9
Ketones in milk or blood	Results due 2020

ABV for energy balance

The MIRforProfit team is also investigating the feasibility of selecting for improved energy balance that will enable farmers to breed for improved resistance to ketosis.

Two ABV predictions have been developed for comparison, one based on the MIR spectra linked to direct energy balance data and the other based on MIR spectra linked to milk fatty acid (C18) levels.

Heritability and accuracy are important indicators of whether a breeding value under development is promising. Early results suggest that both predictions (energy balance and C18 fatty acid) have very high heritability (see table).

The traits currently evaluated by DataGene that have the highest heritability are stature (0.4) and production (0.3). This means that 40% of the variation in stature can be attributed to genetics.

MIRforProfit early results Table 3: ABVs for new traits		
Trait	Heritability	Accuracy
Energy Balance (Ellinbank herd)	0.5-0.6	0.21
Milk fatty acid (C18)	0.45	0.3-0.4

Next steps

Initial work by the MIRforProfit team drew on detailed data collected from the Ellinbank research herd and five commercial herds.

The next phase of MIRforProfit will focus on increasing the accuracy of MIR predictions associated with energy balance. In spring 2017, the MIRforProfit team will collect milk samples for MIR analysis from 10,000 cows in commercial herds. This data will be important for validating and refining the prediction equations developed so far.

The key to using MIR data is having a prediction equation that can be applied nationally to calculate an ABV. Developing a prediction equation requires a reference population that has detailed genetic information (genotype and pedigree) and performance data (phenotype). The more performance data that reaches the genetic evaluation system, the more reliable the ABV. Milk MIR has the potential to be a valuable source of performance data on traits that have been previously difficult or impossible to collect performance data, eg ketosis and other health traits.

Looking ahead: what's possible

Milk MIR is potentially a powerful tool because the turnaround time back to the farm is fast, enabling earlier identification of issues and quicker intervention. This has benefits for at both the individual cow level and at the herd level.

For individual cows, milk MIR results would enable the manager to identify individuals with sub-clinical disease that otherwise wouldn't have been picked up – for example those invisible cases of sub-clinical ketosis that show no obvious signs but have lower production, fertility and overall health. Early diagnosis allows early treatment which is associated with better outcomes and fewer complications.

Milk MIR also opens up the opportunity to shift the focus away from treating individual clinical cases to managing at the herd level. Animal health, welfare and productivity all benefit from a herd-level approach.

Monitoring energy balance from milk samples also opens up opportunities to assess the success of interventions such as changes to the diet.

Potential to monitor other health issues

The promising results to date suggest there is potential for the future development milk MIR tools to monitor for several health problems that are typically difficult to measure objectively. Although further research is needed, it's possible that herd test reports could help to answer questions such as:

- How successful was the transition ration?
- What % of animals are in negative energy balance?
- What % of animals are being fed insufficient protein?
- What % of animals have sub-clinical milk fever?
- Do we have a ruminal acidosis problem?
- What % of animals are cycling?
- What % of cows have inflammatory disease?
- Do we have a disease outbreak?

Being able to monitor a specific health problem through milk MIR opens up the opportunity to develop a breeding value for it. Health traits typically have low heritability and require thousands of performance records to make genomic predictions. Milk MIR offers a feasible way to collect many records with little additional effort or cost to the farmer or industry. It's possible that sometime in the future it may be possible to diagnose uterine infection or endometriosis from a milk sample, or to develop a breeding value for them.

Note: these are ideas on what's possible. Currently there is no work development work underway for these traits.

Methane emissions

Methane emissions from dairy cows are of interest from both an environmental and production perspective.

Methane emissions from cattle contribute to greenhouse gasses so having cattle that emit less methane will reduce their environmental footprint.

Also, cows that emit high levels of methane tend to eat more and may have lower feed conversion efficiency.

Monitoring methane emissions is technically challenging and requires specialist research facilities. MIR technology has the potential to enable farmers to monitor methane from milk samples, in individual cows and across the whole herd. The results could be used to adjust the diet to reduce methane emissions.

Milk MIR could also be used to provide performance data to contribute to the Feed Saved ABV.

Early results indicate milk MIR has good prediction accuracies for methane emissions, at about 0.7.

Thank you

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- Dr Sally-Anne Turner, DairyNZ
- Dr Tim Luke, DEDJTR
- Dr Jennie Pryce, DEDJTR
- Dr Tingting Wan, DEDJTR

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- Colin and Erina Thompson, Cowra, NSW

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Further reading on ketosis

Ketosis; Dairy Australia website. www.dairyaustralia.com.au

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