Practical application of ketosis and energy deficit milk MIR spectral predictions

L.M. Dale[1], K. Droessler[1], F. J. Auer[2], M. Mayerhofer[3], A. Köck[3], C. Egger-Danner[3], F. Onken[4], H. Spiekers[5], P. Hertel-Böhnke[5], E. Stamer[6], A. Werner[1]

[1]Regional association for performance testing in livestock breeding of Baden-Wuerttemberg, Heinrich-Baumann Str. 1-3, 70190 Stuttgart, Germany
[2]Regional association for performance testing in livestock breeding of Austria (LKV - Austria), Dresdner Straße 89/19, A-1200 Wien, Austria
[3]ZuchtData EDV-Dienstleistungen GmbH, Dresdner Str. 89, 1200 Wien, Austria
[4]German Association for Quality and Performance Testing e.V., Irmintrudisstraße 15, 53113 Bonn, Germany
[5]Institute for Animal Nutrition and Feed Management, Bavarian State Research Centre for Agriculture (LfL), Prof.-Dürrwaechter-Platz 3 85586 Poing-Grub, Germany
[6]TiDa, Tier und Daten GmbH, Bosseer Str. 4c, 24259 Westensee, Germany
Corresponding Author: awerner@lkvbw.de

Abstract

During last 15 years different researchers were developing new calibration models linked to milk main components such as fatty acids or minerals, milk biomarkers such as ketone bodies in milk or inflammation indicators or complex components such as energy deficit, ketosis, mastitis, CH_4 or pregnancy with the help of mid-infrared (MIR) spectrometry spectral data. A major provider of MIR services is European Milk Recording (EMR), an umbrella organisation created by former OptiMIR milk recording organisations (MROs), which offer MIR standardisation and predictions. EMR's members are continuously supporting the creation and maintenance of MIR models by collaborating and participating in research projects. MROs having access to milk MIR spectra and prediction models have been increasingly integrating these predictions into services for dairy farmers. Ketosis is a metabolic disorder in ruminants caused by extremely negative energy balance (EB) in early lactation. It may induce an increase in milk fat percentages and ketone bodies as well as a rapid decrease in milk yield, body weight and feed intake. It is also known for causing secondary diseases and fertility problems. Ketosis risk and negative EB can be determined on cow level by using ketone test kits, blood analysis or by milk MIR predictions such as BHB or acetone.

The new idea of KetoMIR was the modelling of ketosis risk based on ketosis diagnosis from veterinaries as reference and milk MIR spectra absorptions as input in order to provide a better indicator in the milk recording service. The first implementation was KetoMIR1 developed by LKV Baden-Württemberg (LKV B.W.). It was based on milk components predicted from standardised milk MIR spectra and is routinely applied by the MROs LKV B.W. and LKV Austria since 2015 respective 2017.

It has to be underlined that until then in literature no information could be found of direct prediction of ketosis risk based on routine MRO spectral and diagnosis data. Since 2018 KetoMIR2 is developed in both MROs within the D4Dairy project. It is a logistic regression model based on standardised milk MIR spectra, sampling moment, lactation number and breed as input and veterinary diagnosis as ketosis reference. The spectra have been pre-processed following the OptiMIR/EMR procedure and corrected for days in milk by Legendre polynomials.

The optimal selection of input parameter was done by using the glmnet R package with lasso method and 10 fold CV. Applied to an external validation set of 11 representative farms the model showed good specificity (0.84) and medium sensitivity (0.72). The KetoMIR2 risk probability shows high correlation with energy deficit, blood BHB and milk yield. KetoMIR2 provides three classes of ketosis warning such as not, moderately and severely endangered and can also be used in herd management to detect general feeding deficiencies. Currently KetoMIR2 is evaluated by feeding advisors of LKV B.W. in a monthly EB report called EMIR. Here it is contrasted with other EB MIR predictions like KetoMIR-1, EB-NEL (as developed by LKV B.W., DLQ and optiKuh) and fatty acid groups. At LKV Austria KetoMIR-2 is being evaluated additionally with blood ketotest kits sampled in 2020.

Keywords: KetoMIR, ketosis risk, ketosis detection, energy balance, NEL, early lactation, MIR milk spectra, dairy cow, dairy farming, optiKuh, D4Dairy, EMR.

Introduction

Ketosis is the number one problem in early lactation. In this phase, up to 6 weeks, a greatly increased energy requirement for milk production dominates. The effect is additionally reinforced by breeding for higher performance. The energy deficit is covered by mobilizing body fat. This leads to an increase in long-chain, mainly unsaturated fatty acids, which are summarized under the term “Preformed Fatty Acids”, and unesterified fatty acids (NEFA). In addition, the production of short-chain and medium-chain fatty acids, the de-novo fatty acids, is falling. In extreme cases, this leads to an overload of the liver, there is an accumulation of ketone bodies in the blood as well as in urine and milk. These are mainly acetone, beta-hydroxybutyrate (BHB) and citrate (Grelet *et al*., 2016). The classic detection methods are an increased fat-protein quotient above 1.5 in dairy breeds and 1.4 in Simmental cattle. A laboratory test of the BHB concentration in the blood is used as a reference finding; here the limit values are 1.2 to 1.4 for subclinical cases and 3 mmol for clinical cases.

BHB can also be measured in milk and urine; the limit values here are 0.2 and 0.5 in milk and 4 mmol/l in urine. In the meantime, inexpensive rapid tests for blood, milk and urine are also available here. Acetone is also an indicator, the greatly increased concentration in breath, milk and urine leads to the typical fruity-sour smell. There are test methods for milk, the limit values are 0.25 or 2 mmol/l. Acetone is highly volatile, so the remaining concentration of the milk sample in the laboratory is often at the detection limit. Even severe weight loss can be used as an indicator if measured regularly. The new concept of KetoMIR is to offer a ketosis risk via milk recording samples that is more precise than e.g. the fat-protein quotient. With each milk recording sample, the farmer receives a herd screening which he can supplement with the above-mentioned methods. Participation in the OptiMIR project resulted for LKV B.W. and later also for LKV Austria in new possibilities of using MIR spectral data. IT processing and standardization was introduced in 2012.

Algorithms for milk MIR components were further or newly developed, e.g. for fatty acids, minerals, BHB, acetone and citrate and are now available. The French tool CetoMIR, based on BHB and acetone, also showed the possibility of ketosis prediction based on MIR spectra. From around 2011, the voluntary collection of veterinary diagnoses, including ketosis diagnoses, has since been started on approx. 1,200 companies based on an 86-part, simplified key based on the model of the Austrian project. The KetoMIR concept consists of the combination of MIR spectra and veterinary diagnoses to predict the risk of ketosis and was the first known work in this direction at the time. Several parameters and concentrations of the above-mentioned MIR components were used. The development began at LKV B.W. 2014, built on 3 years of data availability and went into production at the end of 2015 (Werner *et al*., 2019). The milk analysis with the help of MID infrared spectra (MIR) is a fast and inexpensive way to examine milk recording samples and tank milk samples on a large scale for the main component fat, protein, lactose and urea.

The milk sample is screened by an infrared beam in so-called FTIR analysers and the respective concentration of the substances is calculated using the absorption spectrum obtained and algorithms. The principle is based on the specific interaction of the different wavelengths with the atomic bonds in the milk molecules. With this method, models for fine components such as fatty acids, minerals, lactoferrin (Soyeurt *et al.*, 2009), BHB, acetone, citrate (Grelet *et al.*, 2016), etc. have also been developed over the past 15 years. In addition, models for complex characteristics such as energy deficit (Dale *et al.*, 2019), ketosis (Werner *et al.*, 2019), mastitis, methane emissions and pregnancy could be created. In contrast to the main components, however, a spectrum with long-term stability is required for the latter features. MIR spectra are not uniform and not long-term stable.

The reasons for the variability lie in different implementations of the manufacturers, tolerances of the components even with the same models as well as drift due to wear and tear, climatic fluctuations etc. This requires a correction by a standardization procedure (Grelet *et al.*, 2015). In the standardization process, a distinction is made between manufacturer-specific processes such as those offered in the form of the Foss equalizer and Bentley stabilizer. In the OptiMIR project, a manufacturer-independent process was developed which has been offered by the OptiMIR successor consortium European Milk Recording (EMR) since 2015. In the milk recording samples and milk quality area, the bias slope correction method is used, which determines correction factors using regular pilot samples and reference measurements. Various studies have also shown that EMR standardization can significantly increase the accuracy of the MIR models.

Material and methods

The binomial KetoMIR-1 model for the first 120 days of lactation used two reference classes: “healthy” for milk recording samples from Rind GMON BW farms for which no diagnoses and health-related losses are available. Spectra were classified as “ketotic” or “sick” with a ketosis diagnosis no more than 6 days after the milk recording samples. MIR milk components such as fatty acids, minerals, BHB, acetone, citrate, classic milk recording samples ingredients and fixed effects such as breed, lactation number, lactation week and milking time were used as input parameters. The GLMNET method in R was used to model a logistic regression. A new version of KetoMIR, KetoMIR-2 is currently being developed in the D4Dairy project and made ready for production. The following innovations are used compared to KetoMIR-1. Instead of milk MIR components, the MIR spectral data are used directly, using the pre-processing established in OptiMIR (standardization, 1st derivative, 212 wavelengths).

The integration of the lactation days takes place with the help of Legendre polynomials on the MIR spectra. The basics for KetoMIR-2 were developed in the course of modelling experiments at LKV B.W. already laid at the beginning of 2017. Through the establishment of spectral data processing and standardization at LKV Austria and Zuchtdata as well as EMR membership in 2017, the MIR spectral data and ketosis diagnoses available there were also usable. The collaboration offered the opportunity to create a more robust, cross-population and cross-manufacturer MIR model. For the KetoMIR-2 calibration, data from the period 2012 to 2017 from 10,079 farms with ketosis diagnoses were used. For the milk recording samples with the classification “ketosis”, 1,638 data sets were used with a spectrum of $\pm$ 14 days around the day of diagnosis. For the milk recording samples with the classification “healthy”, 112,545 data sets were used without diagnosis within 60 days of the sample date and without health-related losses. For external test purposes, 11 farms with a high prevalence of ketosis were removed, 4 from LKV AT and 7 from LKV B.W. As with KetoMIR-1, fixed effects such as breed, lactation number and the trial period were included and a logistic regression was modelled using the GLMNET/Lazzo method with 10-fold cross-validation.

Results and discussions

The ketosis probability was calculated for the calibration and test data and the accuracy via sensitivity and specificity was determined using the 0.5 limit values. The model achieved a sensitivity of 0.72 and a specificity of 0.84 in the test. The two-class model was converted for use in a traffic light system with the classes healthy (green), subclinical/endangered (yellow) and clinical/highly endangered (red). Assuming a 20% share of subclinical cases and a 5% share of clinical cases in the first 6 weeks, the new class limits were set at 0.5 and 0.75 using a GMON database for one year. The KetoMIR1 classes were applied to the 2016 GMON annual inventory for evaluation. The representation of the class proportions for the first weeks of lactation shows the expected greatly increased proportion of endangered and highly endangered classifications. One can also see that the dairy breeds are more affected than the dual-purpose Simmental cattle. The representation of the class proportions via the lactation numbers shows that the heifers and cows from the 3rd lactation are more severely affected than the cows in the 2nd lactation. The evaluation of the mean 305-day performance over the KetoMIR classes and lactations gives the following picture. In general, the 305-day performance in the classes “at risk” and “highly endangered” is lower than in the “healthy” class. So, there is already a general, expected depression in performance.

The course of the production mean values in the respective classes over the lactation numbers follows the general course of production that is to be expected for the life of the cows with a lower production in Brown Swiss and Simmental cattle. In the KetoMIR population evaluation, the prevalence of other diseases in the same lactation was calculated based on the KetoMIR classification of the first milk recording result in the classes “at risk” and “highly endangered” relative to the class “healthy”. The value for the diagnosis of cycle disturbance is only slightly higher at 1.2. First calculations and evaluations of genetic correlations of the KetoMIR-1 index showed the potential for use in breeding selection (Hamann *et al.*, 2017).

The final KetoMIR-2 calibration model achieved a mean sensitivity and specificity of 0.76 and 0.84 in the calibration and 0.72 and 0.83 in the external test. A differentiated analysis revealed a sensitivity and specificity for the population groups LKV-AT of 0.72 and 0.81 and LKV-BW of 0.72 and 0.84, respectively. There were also differences for the racial groups. Here an accuracy of 0.76 and 0.83 was obtained for Holstein. For Brown Swiss it was 0.72 and 0.81 respectively, while for Simmental cattle a lower sensitivity of 0.58 compared to a specificity of 0.88 was determined. This is probably due to the lower prevalence of ketosis in Simmental cattle.

In contrast to KetoMIR-1, the cumulative probability curves of KetoMIR-2 showed a very different shape over the lactation weeks. The procedure for defining the threshold values for the traffic light classes therefore had to be adapted. An extensive Pearson correlation analysis of the KetoMIR-2 probability against classic MLP results and the concentration of new MIR ingredients was carried out. Pearson Correlation to the FEQ was 0.46, the MIR predictions with a closer relationship to energy deficit and ketosis resulted in significantly higher values: e.g. for energy balance NEL -0.79; Blood BHB 0.6; Blood NEFA 0.79; Blood glucose -0.76; Acetone in milk 0.65; C18_1CIS9 as the monounsaturated fatty acids 0.73; C12 -0.44, sodium 0.35. This fits well with the known physiological processes and speaks in favour of KetoMIR-2 as an improved alternative to KetoMIR-1. A need for optimization is still seen in the definition of the class limit values. The KetoMIR1-Index has been integrated in the online herd manager in section “Metabolism” at LKV B.W. since 2015 as well as at Zuchtdata/LKV-Austria since 2017.

In the “Overview” mask, the historical course of the class shares at herd level as well as the classification at animal level is displayed for the last 12 milk recording results. Flock shares in the class “endangered” over 20% are coloured yellow, shares in the class “highly endangered” over 5% are coloured red. Shares in the class “healthy” over 80% are highlighted as green. For each testday the KetoMIR-1 index is calculated and displayed to our breeders, advisors and farmers. In the table “Overview of the control year”, the percentage class shares for the test year are shown and highlighted in colour according to the scheme described above. If you follow the link to the trial date, the KetoMIR classes are displayed in the traffic light system for each animal together with the milk recording results.

The KetoMIR classification can be checked against diagnoses, observation, calving, occupancy, the milk recording results, etc. via the link with the animals to the event list. In the table “Overview of the control day” you can find the known KetoMIR herd proportions with a traffic light. After more than 4 years of routine use, LKV B.W. the following conclusion can be drawn. KetoMIR was well received by the companies and consultants, late or missing results are usually asked for immediately. KetoMIR is used by the feeding consultants to assess and adjust the feeding situation. However, KetoMIR does not replace the veterinarian and the direct employment of the farmer with the individual animal and the herd. There was developed at LKV B.W. an additional milk recording report called E-MIR that focuses on the presentation of the energy balance in the herd. E-MIR integrates several MIR parameters: Energy balance EB-NEL (GfE, 2001) from the cooperation of DLQ, optiKUH and LKV B.W., the fatty acids from the EMR/OptiMIR-RobustMilk cooperation grouped into De-Novo and Preformed (FA), the energy detect classification (ED), developed by CLASEL as part of the OptiMIR project, as well as the KetoMIR classes and probability values. The KetoMIR-2 predictions are currently evaluated and fine-tuned.

The report is created in two editions, an edition for the farmer with initially less information, limited to EB-NEL and KetoMIR-1 class, as well as an extended version for LKV advisors and consultants with additional EB-NEL, KetoMIR-1and KetoMIR-2, FA and ED. The farmer receives an animal list with milk recording results supplemented by the energy balance NEL and the KetoMIR-1 classes, sorted in ascending order according to lactation days. Cows are grouped according to lactation period as cow status (primipar F and multipar K). For the KetoMIR classes, the percentage group shares are shown again and highlighted in colour according to the traffic light system. The energy balance is shown against the number of lactation days using a point graphic with a distinction between primipar and multipar. The points within the first 120 days are also stored with the KetoMIR traffic light colour.

To make it easier to classify the farm, population means are grouped according to breed, lactation week, cow status, probationary period and probation month and based on this, a green estimation curve is calculated and integrated into the graph with green colour, separated according to primipar and multipar. It can be clearly seen the typical course from negative EB values at the beginning of lactation to positive values in the middle and before the dry cattle phase. The advisor’s list of animals also contains the percentage of the de-novo and preformed fatty acid groups. The De Novo group is calculated as the sum of short-chain and medium-chain fatty acids, C6 - C14. The preformed group is formed as the sum of the long-chain fatty acids, C17 + C18. In addition, the ED class is output as the energy balance parameter, which is a classification algorithm which marks animals with an extreme undersupply (-) and oversupply (+) relative to the farm average at herd level.

For the KetoMIR values, the KetoMIR-2 classification is also output as well as the respective KetoMIR probabilities as a value between 0 and 1 in order to be able to better recognize class crossers. The E-MIR table with herd averages for the LKV advisors has been expanded to include mean values for fatty acid groups and KetoMIR-2 class proportions. The graphics in the LKV consultant view have been expanded to include a separate representation of the fatty acid groups De-Novo and Preformed, based on the principle of the EB graphics. At the beginning of lactation, long-chain fatty acids from body fat mobilization dominate, the proportion from neogenesis is reduced. In the middle of lactation, things turn around. Towards the end of lactation, the preformed proportions increase slightly due to the reduced feeding and the neogenesis proportions slightly decrease.

Conclusions

Planning for 2021 foresees a strong expansion of the range at LKV B.W. in the area of MIR parameters. MIR technology continues to be viewed as a key technology. The information base is continually being expanded to further optimize herd management, notably through new reports such as Energy-MIR (E-MIR) and udder health-MIR (MastiMIR). It is also planned to integrate other MIR parameters in the herd manager, for example energy balance, fatty acids, ketone bodies, methane, MastiMIR, etc. Regarding the E-MIR report, an evaluation by LKV consultants and continuous correction has started in 2020. The following questions must be answered: Do the results correspond to the situation found? What is the influence of the composition of the food (eg fatty acid additives, protected fatty acids)? Should the presentation and limit values be optimized? Can KetoMIR-1 be replaced with KetoMIR-2? In addition, interpretation and action instructions should be developed for optimal use of the E-MIR. The usability of KetoMIR's results in breeding will also be explored in more detail.

Acknowledgments

KetoMIR2 has been developed in the international big data project D4Dairy – P2.2 Disease Detection with Milk Spectral Data (*https://d4dairy.com/en*, 2018 - 2022). Within this project the model will be further evaluated and optimized for use in routine herd management and breeding.

The EE, FE and EB NEL and ME work was part of the collaborative project optiKuh, funded by the German Federal Ministry of Food and Agriculture. 10 years (2011-2021) of spectral standardization. This work was concepted by CRA-W, founded by OptiMIR project with the support of INTEREG IV B and it is under enhancement and continuous development of EMR-EEIG.

Dale L.M., A. Werner, H. Spiekers, P. Hertel-Böhnke, E. Stamer, F. Gollé-Leidreiter, M. Au, F. Onken. 2019. Prediction of evaluated energy balance (NEL and ME) in dairy cows by milk mid-infrared (MIR) spectra. ICAR Technical Series 2019: 137-141

GfE. 2001. Empfehlungen zur Energie- und Naehrstoffversorgung der Milchkuehe und Aufzuchtrinder. Heft 8, DLG-Verlag Frankfurt a.M.

Grelet, C., J.A. Fernández Pierna, P. Dardenne, V. Baeten, F. Dehareng. 2015. Standardization of milk mid-infrared spectra from a European dairy network. J. Dairy Sci., 98(4): 2150-2160.

Grelet, C., C. Bastin, M. Gelé, J.B. Davière, M. Johan, A. Werner, R. Reding, , J.A. Fernandez-Pierna, F.G. Colinet, P. Dardenne, and N. Gengler. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, beta-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. J. Dairy Sci., 99(6): 4816-4825.

Hamann, H., Werner, A., Dale, L., Herold, P. 2017. Genetic analyses of ketosis and a newly developed risk indicator in Fleckvieh, Braunvieh and German Holstein. ICAR Technical Series no. 22, 105-108.

Soyeurt, H., D. Bruwier, J.-M. Romnee, N. Gengler, C. Bertozzi, D. Veselko, P. Dardenne. 2009. Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry. J. Dairy Sci., 92(6): 2444–2454.

Werner A., F. Gollé-Leidreiter, K. Droessler, F. J. Auer, M. Mayerhofer, A. Köck, C. Egger-Danner, L.M. Dale. 2019. “KetoMIR2” - modelling of ketosis risk using vets diagnosis and MIR spectra for dairy cows in early l