Evaluation of an early warning system for elevated ß-hydroxybutyrate and non-esterified fatty acid values based on Fourier transform infrared spectra from routine milk samples

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Abstract

The objective of our study was to evaluate an early warning system for the detection of elevated ß-hydroxybutyrate (BHB) and non-esterified fatty acid (NEFA) levels in Fourier transform infrared (FTIR) spectroscopy data from routine milk samples. Starting from the monthly milk performance test of the German Dairy Herd Improvement Associations (DHIAs), we evaluated the benefit of more frequent milk sampling in early lactation to detect cows at risk for hyperketonemia and exaggerated fat mobilization. For the validation of the early warning system, milk and blood samples as reference data were obtained from Holstein-Friesian (HF) and German Simmental (GS) dairy cows in a one-year field trial. To establish an early warning system that utilizes a prediction model for FTIR data, the preferable day in milk (DIM) and a suitable sampling interval were investigated. For elevated NEFA values, a DIM of 6 – 13 was identified as the period for preferable sampling. A weekly testing frequency was used for nearly all of the cows in early lactation, and the number of identified cows with elevated NEFA or BHB values was three times higher than the actual situation of milk testing. Prediction models based on the regression tree full model selection (rtFMS) method, as presented by previous work, were validated to detect elevated BHB and NEFA values in FTIR data from routine milk samples. Different model options were compared in the regression tree regarding their significant impact on the prediction performance, measured in balanced accuracy. The chosen prediction model for each metabolite was validated on the reference data set as the gold standard. The evaluated early warning system might be implemented as an additional flexible milk sampling in the routine processes of the milk performance test of the DHIAs.

Keywords: NEFA, BHB, hyperketonemia, Holstein Friesian, German Simmental, prediction model, herd health monitoring

Intoduction

After parturition in cows, the shift from an anabolic state to a catabolic state with the beginning of lactation represents a metabolic challenge. The effective dry matter intake is lower than the nutrient requirements at the beginning of lactation, such that a calculated negative energy balance occurs. Therefore, cows mobilize fatty acids stored in adipose tissue and produce ketone bodies as sources of energy [1]. Subsequently, elevated ß-hydroxybutyrate (BHB) and non-esterified fatty acid (NEFA) values in blood occur. The gold standard thresholds for BHB and NEFA based on photometric measurement in blood serum are 1.2 mmol/l and 0.7 mmol/l, respectively [2–5]. Hyperketonemia (HYK), also named subclinical ketosis and defined in the literature as blood BHB values between 1.2 and 3.0 mmol/l without clinical symptoms, is a widespread problem in dairy cows [4]. Suthar et al. [4] revealed an HYK prevalence of 21.8% in dairy cows in Europe. Multiparous cows with high milk production are more likely to develop ketosis than are those with lower milk production [6].

Tremblay et al. [5] found that the impacts of elevated NEFAs on cow me-

tabolism had been underestimated and were more strongly associated with clinical signs than BHB values [5]. Elevation of both metabolites is followed by health-related and financial risks for cows and farmers, respectively. Cows that suffer from exaggerated fat mobilization and HYK have an economic impact on dairy farms, with a reduction in milk yield and a higher risk for other production diseases [4, 7]. Early detection of elevated metabolites is required to prevent negative consequences. A suitable method for detecting substances in milk samples is Fourier transform infrared (FTIR) spectroscopy. This method is based on the difference in absorption of IR wavelengths in substances. Based on FTIR spectroscopy, prediction models have been developed to identify cows at risk of developing metabolic problems. Chandler et al. [8] constructed a predictive model for serum BHB by using test-day milk samples and performance variables, e.g., breed, parity, and days in milk (DIM). They recommended this model for routine testing, but the high rates of false positives make additional testing in suspicious cases necessary [8]. In a study with German Simmental (GS) cows, Tremblay et al. [9] developed an NEFA and BHB prediction model based on a regression tree full model selection (rtFMS) approach. This approach is a suitable method for generating prediction models that are customized for individual variables and model options [9]. The German Dairy Herd Improvement programme (DHI) covers 88% of the German dairy farms with their monthly milk recording test. We evaluated the application of a prediction model based on rtFMS on the routine processes of DHI. We validated the routine test frequency and investigated the period in lactation with the highest prevalence of elevated BHB and NEFA values. We hypothesized that an extension of the routine milk performance test with an additional test date and the implementation of the verified prediction model for elevated BHB and NEFA values would improve the early detection of cows with elevated BHB and NEFA values. Our objective is the validation of a prediction model regarding its performance and suitability to become a routine screening tool for elevated BHB and NEFA values. We aimed to determine a certain period in lactation and an efficient frequency of sampling to achieve a screening tool for metabolic imbalances.

Materials and Methods

Data collection: For the FTIR data set, milk samples were collected weekly from DIM 5 to 50 from 2,678 dairy cows ranging over a 52-week period starting January 2018. Due to the distributed calving over the year, 64 cows were represented in two consecutive lactations within these 52 weeks and were sampled in both lactations. This resulted in the examination of a total of 2,742 lactations of the included cows. For clarity, we refer to “cows” instead of “lactations”. A reference data set consisting of corresponding blood samples was set up as the gold standard to validate the FTIR data set. Two farms with HF cows in Thuringia took part in the field trial. A total of 2,135 cows were sampled once a week during their normal milking in conventional milking parlours. Eight farms with GS cows in Bavaria were represented by 607 cows. They used automatic milking systems (AMSs) and connected the milk sample shuttle ORI-Collector (SAYCA Automatizacion, Alcalá de Henares, Spain) for 12 – 24 h once per week. The milk sample was branched from the normal milking, that is, voluntarily. Sampling bottles of type 6845-xx (Bartec Benke GmbH, Gotteszell, Germany) containing 2 ml of preservative gel consisting of < 4% sodium azide, < 3% bronopol (2-bromo-2-nitropropane-1,3-diol), and < 0.2% chloramphenicol were used for collecting the milk samples. The samples were transported at 4 °C to the laboratories of the Bavarian Association for raw milk testing (Milchprüfring Bayern e. V., MPR) for FTIR analysis. Infrared spectroscopy of milk samples was performed using the IR spectrometer MilkoScan™ 7 RM (FOSS GmbH, Hamburg, Germany). The milk FTIR absorption spectra were measured and used to derive the milk components, including fat, protein, lactose, urea, BHB and NEFAs. Fossomatic™ FC (FOSS GmbH, Hamburg, Germany) was used to determine the somatic cell count. Blood samples were collected by the investigators the day after milk samples were taken. The Precision Glide™ Vacutainer System with Multi-sample Needles (20G x 1.5”; Becton Dickinson, Franklin Lakes, United States) was used to collect blood from the vena coccygea, and BD Vacutainer® SST II Advance tubes with serum separator (8,5 ml; Becton Dickinson, Franklin Lakes, United States) were filled with 8 ml of blood. After a 30 min coagulation period, the tubes were centrifuged for 10 min at 2,000 G on the Bavarian farms and for 5 min and 20 s at 3,000 G on the Thuringian farms using portable centrifuges. The blood samples were transported at 4 °C to the laboratory of the Clinic for Ruminants in Oberschleissheim. All blood samples were analysed on a Cobas® c311 analyser (Roche Diagnostics, Mannheim, Germany) to obtain BHB and NEFA values in mmol/l. Data sets consisted of BHB and NEFA values in blood and milk and were supplemented with cow data from the milk-record database of the Dairy Herd Improvement Association of Bavaria (LKV Bayern). The cow data present information such as ear tag number, birth date, breed, farm number, current calving date and number and, if necessary, the exit date. Additionally, day in milk, sampling date and time corresponding to each milk sample were added.

Data processing: The original dataset contained 10,776 blood samples. After restricting the data set to samples from HF and GS cows and omitting samples taken outside DIM 5 to 50, 10,474 samples comprised the reference data set. The original FTIR data set of 18,098 milk samples was cleaned by deleting observations with missing input variables to achieve a data set that provided the same features. The selection of 13,472 samples comprised milk FTIR spectra, fatty acid panels, serum BHB and NEFA values, cow information and the presence of standardized IR spectra. The removal of samples missing a corresponding blood or milk value resulted in 11,822 data points. Missingness was assumed to occur at random and was associated with technical problems, frozen sample shuttles or failure to transport at 4 °C, among other issues. Data selection for the presence of fatty acid panels calibrated by Qlip B. V. (Zutphen, Netherlands) resulted in 10,876 data points, and the selection for HF and GS breeds and DIM < 50 resulted in a final data set of 8,459 observations. We evaluated the sum of the individual observations of the cows on a certain date and observed the trend of these results over the course of the one-year field trial. We did not report thresholds at the herd level or compare herd-level results.

Contemplation of prevalence in different aspects: Cut-off values of ≥ 1.2 mmol/l for serum BHB [3, 4] and ≥ 0.7 mmol/l for serum NEFAs [2, 5] were chosen to identify HYK and exaggerated fat mobilization. The prevalence was calculated by dividing the number of samples above those thresholds by the total number of samples. The sampling prevalence for each outcome value was separately split for every lactation week for the HF and GS breeds and for every day in milk. Additionally, the prevalence for cows with at least one sample above the BHB or NEFA thresholds was calculated.

Calculation of different sampling intervals: The milk performance test is generally performed on eleven sampling dates within one year and on average with a five-week interval between the samplings. To identify the benefit of additional sampling dates in early lactation, the present study implemented a weekly sampling interval over 52 weeks. To calculate the effect of different sampling intervals on the number of detected

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cows, we simulated two-, three-, and four-week sampling intervals. To validate the results, the mean values were used. By counting the actual samples with elevated BHB and NEFA values in the reference dataset, we were able to calculate the additionally detected samples above the cut-offs for the different sampling intervals. Subsequently, the number of cows that had at least one sample above the thresholds and were not detected due to the increased sampling intervals was also counted. The number of cows that were not detected with a five-week sampling interval was a main question. We calculated the factor of increased detection for weekly, two weekly, three weekly and four weekly testing, starting from the actual five weekly testing with factor 1.

Prediction model according to the regression tree full model selection method: The rtFMS method described by Tremblay et al. [9] consists of subsequent steps to develop a prediction model, which is customized for the selected input and output variables, as shown in Figure 1. The models were built in R by using the caret package [10, 11]. First, a model is defined as a combination of one selected option from each of seven decision criteria. These criteria are bundled in three areas: input variables, pre-processing methods and model algorithms. All possible options are presented in Figure 2. The outcome selection for the prediction models are cut-off values of ≥ 1.2 mmol/l for serum BHB [3, 4] and ≥ 0.7 mmol/l for serum NEFAs [2, 5]. The FTIR data were prepared for modelling by application of standard methods, i.e., 10-fold-cross-validation and autoscaling. The model does not take into account that there are repeated measurements per cow. This is mitigated by the fact that there are similar numbers of samples per cow, i.e., there is no large bias for individual cows. The selected options were modelled in different combinations. The performance measure of the prediction models was balanced accuracy. Balanced accuracy is especially useful for measuring the quality of a binary classifier, especially for imbalanced data sets [12]. The regression tree consists of decision nodes

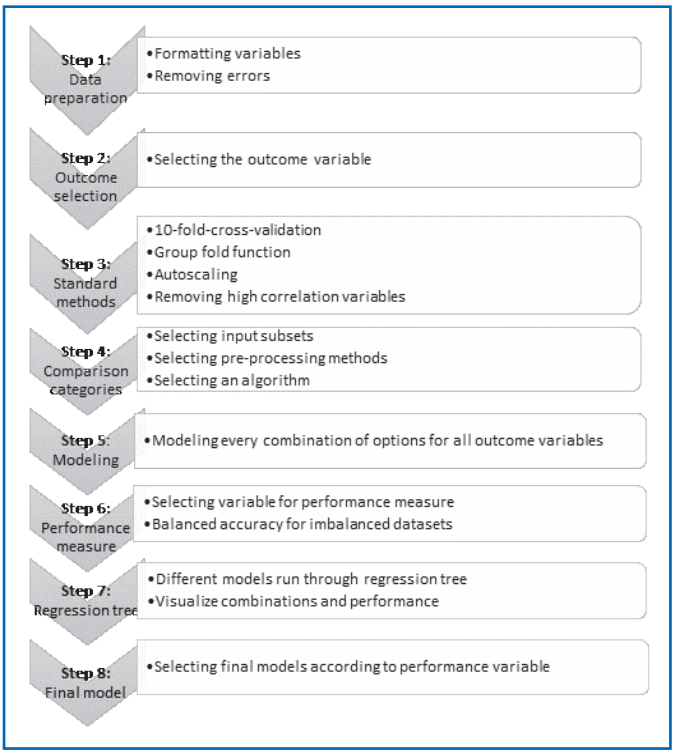

Figure 1: Process scheme for modeling a predictive model with regression tree full model selection (rtFMS) in eight steps based on Tremblay et al. (2019)

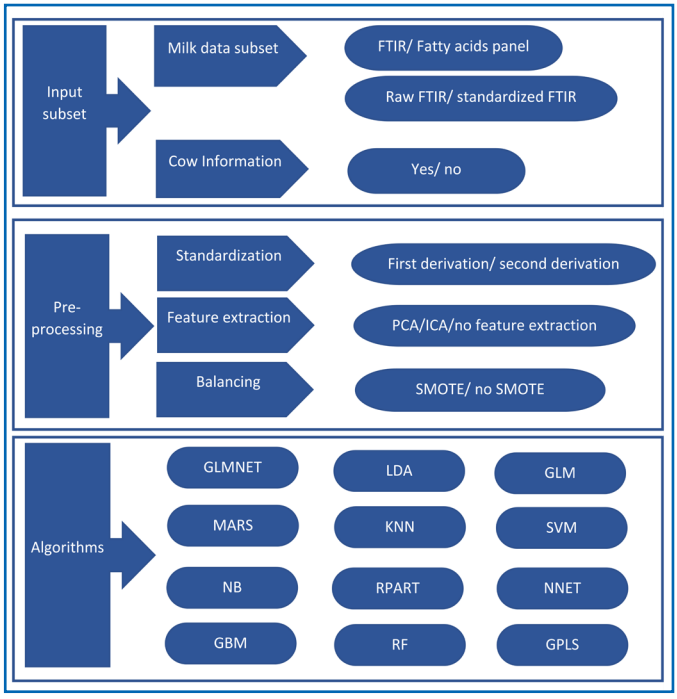

Figure 2: Models are defined as combination of one option from each category. The area (1) Input variables. The decision categories are (1.1) milk data subset with the options (1.1.1) Fouriertransform infrared spectral (FTIR) data, (1.1.2) fatty acids panels, (1.1.3) raw FTIR data, (1.1.4) standardized FTIR data, and (1.2) cow information with the options (1.2.1) Cow information included, (1.2.2) Cow information excluded. The area (2) Pre-processing consists decision category (2.1) standardization with the options (2.1.1) First derivation, (2.1.2) second derivation. And decision category (2.2) feature extraction with the options (2.2.1) Principal component analysis, (2.2.2) individual component analysis, (2.2.3) no feature extraction. And decision category (2.3) balancing with the options of (2.3.1) use of synthetic minority oversampling technique (SMOTE), (2.3.2) no use of SMOTE. The area (3) algorithms consists of the options (3.1) lasso and elastic-net regularized generalized linear models (GLMNET), (3.2) multivariate adaptive regression splines (MARS), (3.3) naive Bayes (NB), (3.4) gradient boosting machine (GBM), (3.5) linear discriminant analysis (LDA), (3.6) k-nearest neighbour methods (KNN), (3.7) recursive portioning for classification, regression and survival trees (RPART), (3.8) random forests (RF), (3.9) logistic generalized linear models (GLM), (3.10) linear support vector machines (SVM), (3.11) neural networks (NNET), (3.12) generalized partial least squares (GPLS)

that compare the prediction performance of the model combinations. The branching identifies the options that have statistically significant ($p < 0.05$) differences in their balanced accuracy. The branching decisions are repeated for each node until the null hypothesis of independence between the outcome selection and the covariates cannot be rejected at a pre-specified level α (α = 0.05). Preference for one of the models from the terminated nodes should result in prediction performances that are statistically indistinguishable at the 95% confidence interval (CI). Furthermore, the performance was evaluated with extensive diagnostic parameters, i.e., sensitivity, specificity, positive and negative predictive value, and likelihood ratio of a positive and a negative test.

Results

Prevalence – samples in total, DIM, cows and breed: The proportion of samples above the cut-off values for BHB was evenly distributed on the lactation weeks, with its highest value of 7.18% between DIM 28 and

Table 1: Samples for Holstein and Simmental above the cut-off values for β-hydroxybutyrate (BHB) ≥ 1.2 mmol/l or nonesterified fatty acids (NEFAs) ≥ 0.7 mmol/l

breed	number of samples NEFA ≥ 0.7	proportion of samples NEFA ≥ 0.7	number of samples BHB ≥ 1.2	proportion of samples BHB ≥ 1.2	number of samples total
Holstein	440	6.2%	374	5.3%	7,089
Simmental	344	10.2%	321	9.5%	3,385
total	**784**	**7.5%**	**695**	**6.6%**	**10,474**

34. The samples with NEFA values above the threshold had a peak of 13.30% between DIM 6 and 13. The results are confirmed by analysing the prevalence for each DIM that was included in the current study. Elevated BHB values occur between DIM 8 and 12, DIM 18 and 20, DIM 32 and DIM 38 and DIM 40 and 42. However, no clear tendency could be identified for the elevation of BHB depending on a particular DIM. Elevated NEFA values occurred on DIM 6 - 14 and DIM 38 and decreased with progressing lactation (Figures 3 and 4). There were no significant differences between the breeds regarding the DIM when the first elevated sample within one lactation occurred. For GS cows, 10.2% of their samples were above the NEFA threshold, and 8.3% of their samples were above the BHB threshold. For HF cows, the proportion of samples with elevated NEFA values was 6.2%, and for elevated BHB values, it was 5.3% of the total of HF samples. Regarding all GS cows, 33.6% and 26.19% of them had at least one sample above the NEFA and BHB cut-off values, respectively, within their lactation. For the HF cows, the proportion above the NEFA threshold was 13.94%, and that above the BHB threshold was 10.69% (Tables 1 and 2).

Calculation of sampling intervals: Compared to a five-week sampling interval of the routine milk performance test, we could detect 1.3 times more cows with elevated BHB and NEFA values with a sampling date every four weeks. At a sampling interval of three weeks, detection was 1.6 times higher for both metabolites. In the case of a two-week sampling interval, the detection was 2.0 (BHB) and 2.2 (NEFA) times higher, and for a weekly frequency, it was 2.9 (BHB) and 3.3 (NEFA) times higher than in the actual five-week interval of the milk performance test. The calculation of longer intervals reveals the proportion of cows and samples that are not covered with sampling in their early lactation period (DIM 5 – 50). We showed that less sampling between weekly and two-week intervals resulted in 5.27% of cows with no sample within a 52-week period. With a three-week interval, 8.83% of cows were missed, and with a four-week interval, 15.13% of cows were not sampled. For the five-week interval, 24.4% of the cows had no sample in their early lactation between DIM 5 and 50.

Selection of prediction models: The modelling of the different option combinations resulted in 329 models for NEFA outcomes and 669 models for BHB outcomes. After the comparison within the regression tree, two final models per outcome variable, with the best prediction performance of all terminated models, were chosen. The final models to predict elevated BHB values are called BHB#1 and BHB#2. BHB#1 uses FTIR spectra, cow information, the synthetic minority oversampling technique (SMOTE), lasso and elastic-net regularized generalized linear models (GLMNET), but no fatty acid panel and no feature extraction are used as options. BHB#2 uses the same options but also uses a fatty acid panel. The final models to predict elevated NEFA values are

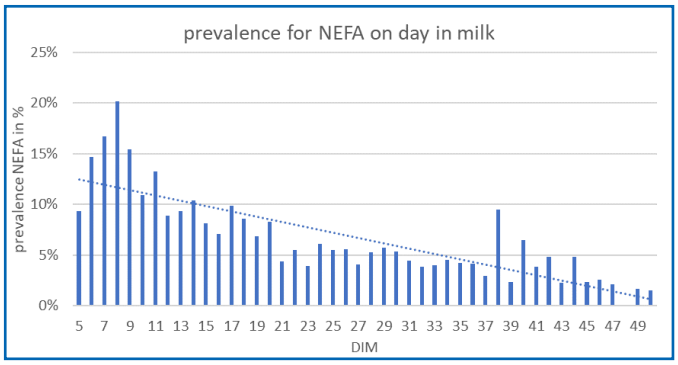

Figure 3: Proportion of samples above the cut-off values for nonesterified fatty acids (NEFAs) ≥ 0.7 mmol/l for each day in milk (DIM)

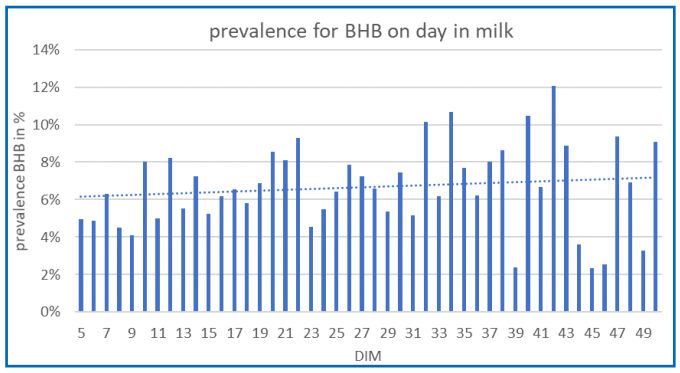

Figure 4 Proportion of samples above the cut-off values for β-hydroxybutyrate (BHB) ≥ 1.2 mmol/l for each day in milk (DIM)

NEFA#1 and NEFA#2. NEFA#1 uses FTIR spectra, fatty acid panels, cow information, principal component analysis (PCA) for feature extraction, SMOTE and GLMNET. NEFA#2 uses the same options excluding PCA. The statistical parameters, especially the performance measure and balanced accuracy, revealed that BHB#2 and NEFA#2 perform better than BHB#1 and NEFA#1, but the CIs of their balanced accuracies overlap. All models could be implemented equally. Statistical parameters and 95% CIs are reported in Table 3.

Discussion

Methods in data collection: We needed to fix the relevant cows for the

Table 2: Holstein and Simmental cows above and beneath the cut-off values for β-hydroxybutyrate (BHB) ≥ 1.2 mmol/l or nonesterified fatty acids (NEFAs) ≥ 0.7 mmol/l

breed	proportion of cows with one or more samples NEFA ≥ 0.7	proportion of cows with no sample NEFA ≥ 0.7	proportion of cows with one or more samples BHB ≥ 0.7	proportion of cows with no sample BHB ≥ 0.7
Holstein	13.9%	86.1%	10.7%	89.3%
Simmental	33.6%	66.4%	26.2%	73.8%
total	18.3%	81.7%	14.1%	85.9%

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Table 3: Diagnostic parameters for selected models of serum β-hydroxybutyrate (BHB) and nonesterified fatty acids (NEFAs) with confidence intervals ≥ 95 % (95 % CI)

Model / Value	BHB#1[1]	95 % CI	BHB#2[2]	95 % CI	NEFA#1[3]	95 % CI	NEFA#2[4]	95 % CI
apparent prevalence %	22.01	21.19 – 22.84	21.48	20.68 – 22.30	21.78	20.98 – 22.61	22.20	21.38 – 23.03
true prevalence %	6.39	5.92 – 6.89	6.39	5.92 – 6.89	7.46	6.95 – 7.99	7.46	6.95 – 7.99
sensitivity %	80.13	76.80 – 83.17	82.18	78.97 – 85.08	77.03	73.82 – 80.01	80.68	72.64 – 83.46
specificity %	81.96	81.17 – 82.74	82.66	81.88 – 83.43	82.67	81.88 – 83.44	82.52	81.72 – 83.29
balanced accuracy %	81.04	78.98 – 82.95	82.42	80.42 – 84.25	79.85	77.85 – 81.73	81.60	79.68 – 83.37
diagnostic accuracy %	81.85	81.07 – 82.60	82.63	81.87 – 83.37	82.25	81.48 – 83.00	82.38	81.61 – 83.12
positive predictive value %	23.27	21.51 – 25.10	24.45	22.64 – 26.33	26.38	24.53 – 28.29	27.11	25.26 – 29.02
negative predictive value %	98.37	98.06 – 98.64	98.55	98.26 – 98.80	97.81	97.46 – 98.12	98.15	97.82 – 98.44
likelihood ratio positive test	4.44	4.19 – 4.71	4.74	4.48 – 5.02	4.44	4.19 – 4.72	4.61	4.36 – 4.88
likelihood ratio negative test	0.24	0.21 – 0.28	0.22	0.18 – 0.25	0.28	0.24 – 0.32	0.23	0.20 – 0.27
number needed to diagnose	0.0062	0.0061 – 0.0064	0.0061	0.0060 – 0.0063	0.0063	0.0062 – 0.0065	0.006	0.0060 – 0.0063
Youden`s Index	0.62	0.58 – 0.66	0.65	0.61 – 0.69	0.60	0.56 – 0.63	0.63	0.59 – 0.67
diagnostic odds ratio	18.32	14.97 – 22.42	21.98	17.81 – 27.13	15.99	13.36 – 19.14	19.70	16.29 – 23.83

[1] BHB#1: Fourier Transform Infrared Spectroscopy (FTIR) data, no fatty acids panel (FA), no standardization, with cow information, second derivation, no feature extraction, synthetic minority oversampling technique (SMOTE), lasso and elastic-net regularized generalized linear models algorithm (GLMNET)

[2] BHB#2: TIR data, FA, no standardization, with cow information, second derivation, no feature extraction, SMOTE, GLMNET

[3] NEFA#1: FTIR data, FA, no standardization, with cow information, with principal component analysis as feature extraction, SMOTE, GLMNET

[4] NEFA#2: FTIR data, FA, no standardization, with cow information, no feature extraction, SMOTE, GLMNET

blood samples and assumed that they experienced stress during this process. Therefore, we took blood samples the day after milk sampling to avoid influencing the milking process and milk samples. Additionally, there were organizational reasons. The voluntary milking in the AMS was the reason that we could receive several samples from one cow within one sampling date. We used the weighted mean for the comparison with the corresponding blood samples. The samples of cows in two consecutive lactations were seen as independent. We handled those samples equal to those from individual cows because they were rare, occurred at random and did not influence the data set.

Prevalence and consequences for detection:

To identify most of the affected cows, it is important to examine the course of elevated BHB and NEFA values within lactation. According to the literature, we expected the early lactation period between DIM 5 and 50 to be the period with the highest prevalence of elevated BHB and NEFA values. The results present cows with NEFA values above the threshold of 0.7 mmol/l blood uniformly between DIM 6 and 13. With an additional out layer on DIM 38. This confirmed the assumption of increased fat mobilization with starting lactation and increased energy requirements. This could be explained by the physiological processes of catabolic metabolism. First, the organism reacts with mobilization of body fat and produces acetyl-CoA in the ß-oxidation of fatty acids. Second, hepatic oxaloacetate limits the use of acetyl-CoA, and they are used to build ketone bodies. The accumulation of ketone bodies in blood is followed by fat mobilization and therefore later lactation [13]. Our findings are similar to those of Tremblay et al. [5] and underscore the NEFA values as more meaningful for early warning systems that detect metabolic imbalances.

Evaluation of impact by breed: The prevalence including all samples of Simmental and Holstein Friesian breeds is 7.18% for BHB and 13.30% for samples above the NEFA threshold. The proportion of the elevated samples of GS cows was 8.3% (BHB) and 10.2% (NEFA) higher than that of the elevated samples of HF cows (5.3% (BHB) and 6.2% (NEFA)). These findings might be explained by the difference in the structure and size of the dairy farms in Thuringia compared to the Bavarian farms. Thuringian farms employ herd managers who are responsible for the continuous monitoring of dairy herds. For example, the detection of increased BHB and NEFA values during the field trial in one of the Thuringian farms caused an examination of their feed. A thorough feed analysis revealed a lack of nutrient value in charge of their hay. After correction for the energy supply, the BHB and NEFA values decreased continuously.

In contrast, the Simmental breed showed a proportion of more cows with elevated NEFA and BHB values than the HF cows. Considering that 33% of the investigated GS cows showed elevated NEFA values at least once in their early lactation, the requirement for an early warning system is obvious. Regarding the affected time, we could not identify differences between the breeds. We reported the first occurrence of elevated BHB or NEFA values on DIM 6 - 13 for NEFA and DIM 7 - 13 and DIM 20 - 22 for BHB. Mc Art et al. [7] reported that the incidence of

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HYK was 43% between DIM 3 and 16, with a peak on DIM 5. Metabolic differences in HF and GS cows were evaluated by Gantner et al. [14, 15]. Their findings present the peak in prevalence for elevated BHB in multiparous HF cows on DIM 25 and in primiparous HF cows on DIM 15 [15]. The prevalence in GS cows occurs in the 1st, 2nd and 3rd parities on DIM 20 and in cows in their fourth or higher parity on DIM 25 [14]. These studies confirmed early lactation, as we sampled in the field trial, as a period of high risk. In contrast, we did not consider samples before DIM 5 to avoid sampling of colostrum, which is not meaningful in FTIR prediction due to its composition.

Investigated sampling intervals: Starting from the actual sampling frequency of the milk performance test, we evaluated the potential of more frequent testing. The validat ion of the prediction model on data of field-gained samples is innovative for the milk performance test. The number of identified cows above the BHB and NEFA thresholds is rising with more frequent testing. The detection of elevated metabolites profits from a shortened sampling interval. The even distribution of the observations offers weekly sampling as the best alternative to represent as many cows in their early lactation as possible. Similarly, the number of cows that were not sampled in their early lactation decreased with more frequent testing. Today's milk performance test covers two-thirds of the cows in their early lactation, but weekly testing is able to sample almost all cows once in their critical period between DIM 5 and 50. In summary, we welcome weekly testing, as this interval could document high values in positive predictions. Subsequently, the avoidance of metabolic imbalances, the prevention of decreasing performance and the maintenance of cow health are strong arguments for more frequent testing. Although the effort taken in our study over a one-year period was not low, the involved dairy farmers gave positive feedback throughout the study due to the accurate determination of the metabolic state of their cows. Additionally, identifying cows before they are affected by metabolic imbalances such as HYK and exaggerated fat mobilization and subsequently the reduction of costs is possible. Classification of herd health by means of the individual BHB and NEFA values of the cows in the herd is also an advantage. However, economic factors and efforts that come along with milk sampling need to be considered, and perhaps a compromise between high-frequency monitoring and economically reasonable sampling needs to be found. We prefer milk samples for routine testing because it is the method with less cow handling and is non-invasive. We experienced in our field trial that the use of the sampling shuttle makes individual cow handling unnecessary. The cows in the relevant lactation period are chosen on the herd manager PC, and the automatic milking system detects the relevant cows while milking and branches a sample of them. In the milking carousel, the relevant cows are handled anyway and can be identified easily. In the case of blood or urine testing, the cow needs to be separated, fixated and sampled. This is an alternative method, especially for already impaired cows or in other suspicious cases. The processes in dairy farms and in their veterinary health care become more digital and automized. Sampling without additional handling would be more innovative. The compliance of the dairy farmers and the possibility for practical implementation on farms are the baselines for realization. The solution could be flexible additional milk sampling in the early and therefore riskier period after calving. The American Dairy Herd Improvement Association, for example, provides different services. Monthly routine testing and further testing for fresh cows from DIM 7 to 13 were invoiced separately. A customer-friendly and individual milk testing service could continuously support the improvement of herd management and the animal health of dairy farms. Thus, the costs and benefits need to be weighed to decide the frequency of routine milk testing that is appropriate to discover metabolic imbalances [16, 17].

Prediction models for routine metabolic monitoring: The promising results of modelling a prediction tool with the rtFMS approach, described and published by Tremblay et al. [9], led to the application of this method on the FTIR data of the current study. The rtFMS approach allows for stepwise modelling strategies based on standardization, different input subsets, pre-processing options and several algorithms. The systematic stepwise rtFMS prevents user bias and improves the prediction model performance by optimizing balanced accuracy. The presented prediction models were evaluated regarding their performance and quality. Therefore, different preconditions were verified for their impact on practical realization. The assumption that fatty acids have a greater impact on the metabolic imbalance, as seen before, led to the decision to add fatty acid panels to the FTIR data. The calculations of the prevalence confirmed that the observations of elevated BHB and NEFA values are rare events with < 15% occurrence in our data set. To level the imbalances, SMOTE was one of the options for modelling, and the regression tree showed significance in the prediction performance for models that use SMOTE [12]. The selected best performing model algorithm in this study was GLMNET, a type of regression model that uses least absolute shrinkage and selection operator (Lasso) and ridge regression. The ability to reduce variables and parameters is especially important in processing FTIR spectral data [18]. The model option to use cow information was also important for the prediction performance. This might be due to the impact that individual key indicators, such as lactation number, day in milk or milk yield in kilograms, decide to adapt to metabolic challenges in dairy cows. The model option of standardization presented no significant impact on the prediction performance due to the use of one FTIR spectrometer and no differences in the calibration of the FTIR data [19]. In our study, the regression tree led to the identification of four final prediction models, two for each outcome variable. The BHB#2 and NEFA#2 models showed better balanced accuracies and better diagnostic parameters than the BHB#1 and NEFA#1 models, respectively, and are preferable if fatty acid panels are available. The BHB#1 and NEFA#1 models are the best alternatives if fatty acid panels are not available and the input data set contains only FTIR data. Using our own data set, we expected to achieve a tool capable of detecting cows at risk for elevated BHB and NEFA values with high accuracy and reliability. The transparency in the regression tree process allows the important options to be identified. The presented prediction model is the technical solution for the proven requirement of early detection of metabolic disorders and routine implementation on dairy farms.

Conclusions

Increased BHB and NEFA values represent a poor adaptation of negative energy balance and are followed by health-related and economic consequences. Therefore, the detection of cows at risk for hyperketonemia (HYK) and exaggerated fat mobilization, determined as elevated BHB and NEFA values, respectively, was the reason for the validation of an early warning system that could be implemented in routine processes. We identified the period in early lactation, between day in milk (DIM) 5 and 50, when prevalence is at its top. In particular, NEFA values showed that DIM 6-13 was a preferable period for additional sampling. The comparison of sampling every five weeks, as in the actual milk performance test, revealed that the number of detected cows with elevated BHB and NEFA is three times higher, with weekly

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sampling. The actual 11 sampling dates over the 52-week period of the milk performance test cover two-thirds of the dairy cows in their early lactation. With a weekly sampling interval, almost all cows are sampled in this period. The next step towards an early warning system was the validation of the technical solution for the detection of elevated BHB and NEFA values in routine processes. The evaluated prediction model was developed with the rtFMS method and applied to FTIR spectra of milk samples and corresponding blood samples of dairy cows in their early lactation. The validated prediction models provide strong prediction performance and high prediction quality, as evidenced by the statistical parameters, i.e., balanced accuracy, sensitivity and specificity. The rtFMS method can meet complex modelling demands and combine many input variables and modelling options with high transparency. The results prepare the implementation of the prediction model as a routine screening tool to strengthen preventive herd health care. We summarize that an extension of the routine milk performance test with an additional test date and the implementation of the verified prediction model for elevated BHB and NEFA values improve the detection of cows with elevated BHB and NEFA values.

Compliance with Ethical Standards

The authors declare no conflicts of interests.

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