Development of an early warning system to predict subclinical metabolic disorders in dairy farms with Fourier transform infrared spectra from routine milk samples

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Meinem Mann Christian und meinen Kindern Juli, Andreas, Mathilda und Pauline

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TABLE DIRECTORY

ABBREVATIONS

AM/PM	Ante meridiem/post meridiem
AMS	Automatic milking system
ANN	Artificial neuronal network
AP III	Working package III
BCS	Body condition score
BHB	ß-hydroxybutyrate
BLE	Bundesanstalt für Ernährung und Landwirtschaft
BMEL	Bundesministerium für Ernährung und Landwirtschaft
CI	Confidence interval
DHI	Dairy Herd Improvement
DHIA	Dairy Herd Improvement Association
DIM	Day in Milk
DLQ	Deutscher Verband für Leistungs- und Qualitätsprüfungen e. V.
FA	Fatty acids
FPR	Fat-to-protein ratio
FSM-IRMI	Frühwarnsystem für Stoffwechselerkrankungen von Milchkühen mit Infrarotspektroskopie der Milch
FTIR	Fourier transform infrared spectroscopy
GLMNET	Lasso and elastic-net regularized generalized linear models algorithm
GS	German Simmental
HF	Holstein Friesian
HYK	Hyperketonemia
IR	Infrared
ITB	Integrierte tierärztliche Bestandsbetreuung
LKV Bayern	Landeskuratorium der Erzeugerringe für tierische Veredelung e. V.
MLP	Routine milk performance test
LKVs	Landeskontrollverbände
LMU	Ludwig-Maximilians-Universität
MPR	Milchprüfring Bayern e. V.
NEB	Negative energy balance
NEFA	Nonesterified fatty acids
PCA	Principal component analysis
PLSR	Partial least square regression
PMAS	Poor metabolic adaptation syndrome
rtFMS	Regression tree full model selection
Se	Sensitivity
SE	Standard Error
SD	Standard deviation
SOP	Standard operation process
Sp	Specificity
SMOTE	Synthetic minority oversampling technique
TSchG	Tierschutzgesetz

I. INTRODUCTION

“These ladies are so hardworking!” (Bavarian dairy farmer, 2018)

The requirements of dairy cows and their farmers have changed in the last decades and equally has the part of the supporting veterinarian. The number of herds has been decreasing, but the number of dairy cows has been rising. Dairy farms have been enlarging and the daily work has become more automated. A broad supply of data is collected by the automatized processes. This information is available to optimize the economic situation and health status of the herd. Although most of the dairy farmers are interested in the health and welfare of their cows, the verification of animal welfare and herd health in dairy farms through self-control is regulated by law (TSchG §11 Abs. 8). Unfortunately, there is no standard process available to define the requirements of animal welfare and herd health nationwide, even though several monitoring and data collection systems are available. Facing this problem, the German Association for Performance and Quality Testing (DLQ) initiated a project to define animal welfare indicators and to implement a nationwide monitoring of animal welfare and herd health. To bring different information systems, data bases, and know-how together and to allow a comprehensive monitoring of herd health and animal welfare, the project Q-Check[1] was set up. It consisted of six interdisciplinary project groups. They worked on different parts of the project and involved the following project partners:

Thuenen-Institute of Organic Farming, Federal Research Institute for Rural Areas, Forestry and Fisheries (Thünen-Institut)

Osnabrueck University of Applied Sciences, Faculty of Agricultural Sciences and Landscape Architecture (Hochschule Osnabrück)

Clinic for Ruminants with Ambulatory and Herd Health Services, Ludwig-Maximilians-University Munich (LMU München)

Dairy Herd Improvement Association of Bavaria (LKV Bayern e. V.)

IT-Solutions for Animal Production (vit)

[1] Tierwohl mit System – von der betrieblichen Eigenkontrolle bis zum nationalen Monitoring
Animal welfare in dairy farming – from self-assessment to national monitoring by system

Bavarian Association for raw milk testing (Milchprüfring Bayern e. V., MPR)

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE).

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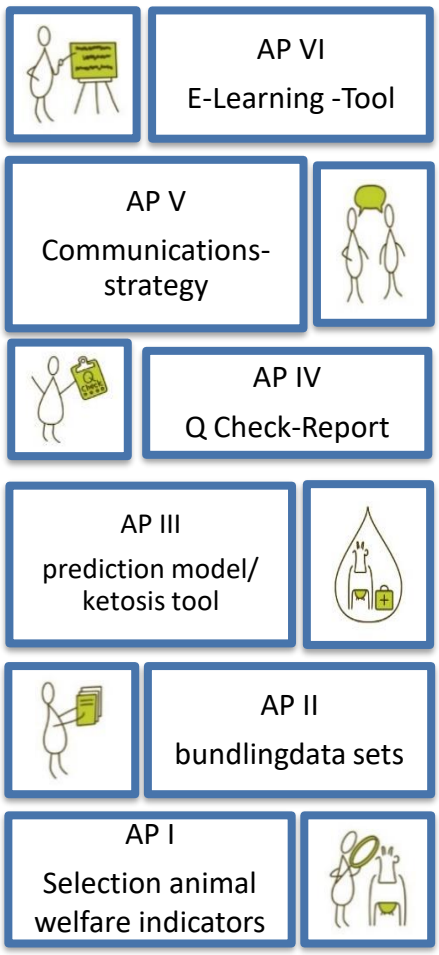

Figure 1 Project Q-Check, interdisciplinary working packages (AP I – VI) (DEUTSCHER VERBAND FÜR LEISTUNGS- UND QUALITÄTSPRÜF-UNGEN E.V., 2020)

According to the main objective of Q-Check, animal welfare and health, the target of the working package three (AP III) was the prevention of dairy cows from metabolic diseases caused by elevated ß-hydroxybutyrate (BHB) and nonesterified fatty acids (NEFA). The Clinic for Ruminants with Ambulatory and Herd Health Services, Ludwig-Maximilian-University Munich, is responsible for AP III. Their main task is the validation of a suitable method to detect metabolic imbalances with high accuracy and before they become clinical. Therefore, a

prediction tool for elevated values of NEFA and BHB in Fourier transform infrared spectroscopy (FTIR) from milk samples was developed and validated. The required preconditions and variables to set up regular herd health monitoring were evaluated and determined. The aim was to develop a tool, that is balanced in effort and benefit and earns compliance by the dairy farmers to convince them to integrate it as a part of their herd health management. Additionally, the tool needs to be applicable by the German Dairy Herd Improvement Associations (LKVs) and be easily implemented in the existing processes of the routine milk performance test (MLP).

To argument the cost and benefit of herd health monitoring and identify its part in integrated veterinary health care (ITB), a literature study was done. This study is the content of publication 1. Afterwards, the development and validation of prediction models started. To evaluate it on standardized FTIR data, a field trial was set up to collect milk and blood samples of approximately 2541 dairy cows in their early lactation for one year. Within the publication 2, the following preconditions and aspects to prepare the implementation of the prediction tool were evaluated.

The main dairy breeds in Germany are Holstein Friesian (HF) cows and German Simmental (GS) cows. The Q-check data set includes both breeds to identify potential differences and allows to compare the quality of the prediction models depending on the breed.

Metabolic disorders mostly occur in the early lactation. To encircle the day in milk (DIM) with the highest prevalence and to determine an optimal sampling frequency, the field trial included cows between DIM 5 and 50 in a weekly sampling scheme of milk and blood samples.

To identify the assumed impact of different daytimes on the results, the samples were taken alternating during the day and at night time.

Compared with cows in lactation two and higher, cows in their first lactation show differences in their metabolism and in milk production. To clarify the impact of these differences regarding the prediction quality, the variable of being heifer or cow was considered.

Due to differences in the milk analyzers of the different laboratories, a standardization of the data set before it was applicated on the prediction models was questioned.

In the first publication, the question why herd health monitoring is reasonable, is discussed and in the second publication the validation of prediction models and their preconditions to realize the herd health monitoring in practice is presented. The overall thesis is to introduce a method, that is suitable to detect metabolic imbalances early and is easy to implement in routine processes to secure the improvement of animal health and welfare.

II. LITERATURE

1. Hyperketonemia (HYK)

Hyperketonemia is a disturbance in the energy metabolism. It is caused by a disparity between energy demand and energy uptake. During the transition period, the metabolism changes from an anabolic to a catabolic situation (RABOISSON et al., 2014). The energy demand increases in the periparturient period by 30 % uo to 50 %. Nonetheless, the actual dry matter intake cannot cover the nutrient demand. A negative energy balance (NEB) is almost unavoidable in the early lactation (BELL, 1995; BAIRD, 1982).

To ensure the energy supply, body fat tissue is mobilized and the liver produces ketone bodies which helps the organism to obtain important body functions and cover the energy gap for a certain time. If the cow is not able to take up enough energy, and the NEB remains, the fat mobilization and the ketone bodies will exaggerate the metabolic capacity of the liver and a metabolic disorder occurs (DIRKSEN AND BAUMGARTNER, 2006; INGVARTSEN, 2006).

The majority of affected cows are subclinically ill and do not show symptoms despite a reduction in milk yield (DUFFIELD, 2000). Subclinical ketosis is defined as an elevation of blood BHB starting from 1.2 mmol/L without clinical signs, whereas clinical ketosis entails an elevation of more than 2.9 mmol/L BHB in blood and clinical symptoms such as reduction in milk production, decreased feed intake and impaired foregut motility as well as a massive loss of body weight. Sometimes, neurological signs such as nervousness, aggressiveness and roaring can be observed (SUTHAR et al., 2013; OETZEL, 2004; BERGE AND VERTENTEN, 2014; BAIRD, 1982). Recent studies prefer the term hyperketonemia (HYK) to describe the metabolic disturbance due to the elevation of BHB values over 1.2 mmol/L.

2. Poor metabolic adaptation syndrome (PMAS)

TREMBLAY et al. (2018), showed a significant correlation of clinical symptoms with the elevation of NEFAs in their study. They introduced the term “poor metabolic adaptation syndrome” (PMAS). NEFA values in blood are increasing with massive mobilization of body fat. A cluster analysis revealed a significant

correlation between cows with higher milk yield, increasing parity, and higher amount of back fat at the beginning of lactation. The authors observed decreased rumen fill, elevated liver enzymes, and elevated NEFA values in affected animals. The threshold to identify cows at risk for PMAS, is 0.7 mmol/L NEFA in blood or higher.

Due to these findings and the necessary change in terminology of metabolic disturbances, hyperketonemia is used as synonym for BHB values ≥ 1.2 mmol/L blood and PMAS as synonym for NEFA values ≥ 0.7 mmol/L blood.

3. HYK and PMAS

3.1. Epidemiology

HYK and excessive fat mobilization in early lactation are well-known problems within dairy production The mean prevalence in Europe has been shown to be 21.8 % compared with 22.6 % in Canada (SUTHAR et al., 2013; SANTSCHI et al., 2016). The peak incidence between DIM 3 and 16 rises up to 43 % and increases with parity (MC ART et al., 2012). Especially subclinical forms have a prevalence of up to 34 % in the first two months of lactation (DUFFIELD, 2000).

3.2. Impact on health and milk production

Although clinical symptoms are not necessarily obvious, the impact on health and performance of the cows is considerable. As a consequence of the elevated BHB and NEFA values, the risk for several production diseases such as displaced abomasum, retained placenta, and metritis dramatically increases. Cows have 3.3 times higher odds to suffer from displaced abomasum and 1.52 times increased odds for retained placenta. The odds ratio to be culled within 60 days after calving for a cow with HYK is 1.92 (MULLIGAN AND DOHERTY, 2008; RABOISSON et al., 2014). High-producing cows are more likely to develop a NEB due to their increased nutrient demands. The milk production of cows with elevated BHB or NEFA values decreases approximately 3.0 - 5.3 kg per day during the two postpartal weeks. For cows in higher lactation numbers, the reduction is more severe than in lower parity (RAJALA-SCHULTZ et al., 1999).
Given associated diseases or reduction in performance, the economic impact on the farmer is obvious. The total costs have been calculated by Mc ART et al. (2015) with $ 289 per case of HYK. The main components are losses in

reproductive performance, losses by death, and losses in milk yield. Assuming the above-mentioned prevalence of 21.8 %, the financial loss for a dairy farm with 100 cows lays between $ 4,425 and $ 6,300 (GRUBER AND MANSFELD, 2019).

3.3. Diagnostic methods

The gold standard for diagnosing elevated BHB and NEFA values is the photometric measurement of these parameters in blood. Due to the requirement of a veterinarian for taking blood samples and related costs and effort as well as the delay through laboratory analysis, it is not suitable for a preventive herd monitoring (OETZEL, 2004). There are also many test kits working with urine, milk or blood, that are inexpensive and provide quick and accurate results. Mostly, they are used for individual and suspicious cases, but are not suitable for routine monitoring (IWERSEN et al., 2009). Several studies recommend FTIR spectroscopy of routine milk samples to gain information of the metabolic status on herd level (DENIS-ROBICHAUD et al., 2014; SANTSCHI et al., 2016). VAN KNEGSEL et al. (2010) have already examined FTIR measurement of BHB and NEFA values with a sensitivity (Se) of 80 % [confidence interval (CI) 65 – 90 %] and a specificity (Sp) of 71 % (CI 68 – 75 %). To prevent cows from HYK and PMAS, an early identification of cows at risk is required. That could be achieved by routine monitoring of the BHB and NEFA values within the herd.

3.4. Prevention with prediction models

To improve the prevention from HYK and PMAS, the objective was to discover cows before being impaired. Prediction models of critical thresholds of NEFA and BHB values allow to detect cows which are at risk to develop metabolic imbalances, if they remain untreated. Various types of prediction models have been described in literature.

GRELET et al. (2016) developed prediction equations by use of partial least square regression (PLSR) for biomarkers such as citrate, acetone, and BHB in milk samples. Their equations are predicting high or low levels of ketone bodies with high accuracy.

Another approach is the evaluation of an artificial neuronal-network (ANN) on metabolic, genomic, and milk-performance data. The prediction model refers to BHB values as outcome variable and combines different model options utilizing

metabolic, milk performance and genetic data. The prediction quality is more accurate when using milk performance as well as metabolic data than in the model with the genetic information. To measure the performance, the Pearson's correlation coefficient was used (HAZRA AND GOGTAY, 2016). On average, a difference of up to 0.643 appears between predicted and observed values if both metabolic and routine milk data are combined (EHRET et al., 2015).

CHANDLER et al. (2018) evaluated prediction of HYK in comparison of HF and Jersey cows. The objective was to compare the accuracy of prediction in linear and logistic regression models and to determine thresholds regarding the correlation between serum and milk BHB and acetone.

The examinations were stratified by breed, Holstein Friesian and Jersey, and parity (first vs. higher parity). The final logistic regression models showed results of a Se up to 55.6 % (CI 50.9 – 60.1 %), a Sp of 99.0 % (CI 98 - 99.2 %) and an accuracy of 97.3 % (CI 96.9 - 97.5 %) for primiparous HF cows. The best performance was in logistic models the one for primiparous HF cows.

The multiple linear regression models predicted with 97.8 % (CI 95.8 - 99.7 %) accuracy, a Se of 56.6 % (CI 23.1 – 86.3 %) and a Sp of 99.5 % (CI 98.6 – 100 %) in primiparous HF cows.

The multiple linear regression model was also assumed to generate accurate herd-level prediction, which was confirmed with 80 % accuracy in HF herds with an alarm level of 10 % prevalence.

CHANDLER et al. (2018) have recommended a multiple linear regression model for predicting HYK in cows as a reliable management tool. Compared with a logistic regression model and thresholds of ketone bodies in FTIR data, the multiple linear regression model shows better performance and prediction quality. However, the authors regarded the limitation of the model to be an overestimated predicted prevalence and high values of false positives. They recommended the multiple linear regression model as monitoring tool for herd-level HYK predictions that result in management decisions regarding the nutrient requirements.

4. Statistical parameters to evaluate prediction models

The performance of prediction models for BHB and NEFA, respectively is presented by selected statistical parameters. Due to their function to measure the diagnostic quality, they are also referred to as “diagnostic parameters” in the publication “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”.

The main parameter to measure the prediction performance in the presented studies was balanced accuracy. This metric is especially useful to measure the quality of a binary classifier, especially for imbalanced data sets.

Balanced accuracy = Se + Sp / 2
Se = true positive / total positive

Sp = true negative / total negative

Diagnostic accuracy = true positive + true negative / total population

Apparent prevalence = positive predicted / total population

True prevalence = true positive / total positive

Diagnostic odds ratio = (true positive / false positive) / (false negative / true negative)

Youden’s Index = Se + Sp - 1

Number needed to diagnose = 1 / Youden’s Index

Positive predictive value = true positive / (true positive + false positive)

Negative predictive value = true negative / (true negative + false negative)

Likelihood ratio of a positive test = Se / 1 - Sp

Likelihood ratio of a negative test = 1 – Se / Sp

III. MATERIALS AND METHODS

1. Literature research and publication

As first approach to the thesis, a literature research was conducted. The objective was to argument the thesis supported by subject-related literature. Therefore, main arguments to support the objective of herd health monitoring were collected and discussed in the publication "Herd health monitoring in dairy farms – discover metabolic diseases. An overview" (GRUBER AND MANSFELD, 2019).

The literature research was conducted with the online services Pubmed, Web of Science, Google Scholar, and the library of the LMU. Literature regarding the metabolic diseases and veterinary aspects was used as well as literature to comprehend the FTIR technology and sources to illuminate economic aspects of herd health monitoring. The efforts and benefits of monitoring strategies were examined and underscored with academic literature. Quoted literature was selected according to academic standards, original research articles and meta-analyses were preferred sources.

The collected literature confirmed the thesis of the requirement of herd health monitoring to support and improve dairy farming and integrated veterinary herd health care.

2. Project coordination and preparation

Before the beginning of the field trial in January 2018, the sampling and logistical processes were prepared. Especially the required animal test proposals were successfully applied. The official number for the animal experiment proposal in the Government of Bavaria is the reference ROB-55.2Vet-2532.Vet_03-17-84, and that at the Thuringian State Office of Consumer Protection is the reference 22-2684-04-LMU-17-101. The equipment and logistics organisation with Qnetics GmbH (Thuringia) and LKV Bayern were completed. Additionally, job interviews for veterinarians to support the blood sampling in Bavaria were conducted. A pre-trial was held up from September 2017 to October 2017 to check the processes determined in the standard operating processes (SOP). During the main trial from January 2018 to December 2018, weekly metabolic reports

and recommendations for veterinarian treatment of elevated cows above the defined thresholds of ≥1.2 mmol/L BHB or ≥ 0.7 mmol/L NEFA, were supplied.

The organisation of the AP III also required the embedding in the Q-Check project with presentation of the project steps and results to the project partners, especially the Q-Check project coordination at the DLQ and the public financiers at the BLE. Presentations of results and project workshops, as well as a monthly conference call were conducted. The validation of the prediction models, developed within the project group in Wisconsin in April 2019, was completed in November 2019. The results were presented on a video conference with the DLQ and the LKVs in June 2020. The Q-Check project had a broad audience, beginning with the milk producers, the veterinarians, the dairy associations, and the public financiers as the BLE. All these groups had to represent their interests in dairy farming and veterinarian herd health care. The coordination between the groups and the representation of veterinarian and academic interests were main tasks for the author within Q-Check.

3. Data collection within the field trial

The dairy farms which took part in the present study were selected by several aspects. Dairy farms in Bavaria are mostly family-run farms with up to 70 cows. Farms with automatic milking systems (AMS) and herds consisting in majority of GS cows were acquired. They were asked to select cows between DIM 5 and 50 in their AMS and took milk samples with a milking sample shuttle type Ori Collector Version Light SD (Sans Systeme Doseur, Alcalà de Henales, ESP) for at least twelve hours once a week over a period of 52 weeks. Additionally, veterinarians needed to be allowed to take blood samples from these cows on the day after the milk sample had been taken. Eventually, eight dairy farms were selected with herds between 56 and 150 dairy cows about 30 km around Munich. In Thuringia, two dairy farms with HF herds were selected for the field trial. The dairy farm in Dermbach had about 1,500 milking cows and the farm in Buttelstedt had about 500 cows. On both farms, the milking system conducted a milking parlor and the sampling was carried out during normal milking, alternating between the morning and evening milking. The main advantage of the two farms in Thuringia was to represent about 2,000 cows within two farms and to consider only two farming systems. They were selected due to their reliability of taking

milk and blood samples always in the same way to avoid deviations through sampling failures. The selection of the farms allowed to present field conditions with different milking systems and farm sizes. Dairy farming in Germany showed a high variety due to regional circumstances. The field trial collected data from several types of dairy farms to represent the metabolic situations within the specific preconditions and aimed to evaluate the prediction models in these different environments. To improve their herd health management by achieving weekly analysis of their cows in early lactation, the dairy farms appreciated the present study.

The samples were analysed by the MPR and the laboratory of the Clinic for Ruminants, and the results were sent to the farmers in a weekly email report. At the end of the trial, the data were completed with cow-level information from the LKV data base, cleaned for missing values, and sent to the project partners at the School of Veterinary Medicine/University of Wisconsin.

The author prepared the field trials with SOP for milk and blood sampling in Bavaria and for each of the dairy farms in Thuringia. SOPs can be reviewed in appendices 1-4.

4. Prediction models based on regression tree full model selection

Based on FTIR spectral data from milk samples of a previous project, TREMBLAY et al. (2019) developed a prediction model, based on the regression tree full model selection method (rtFMS) to compare different model options and to achieve the model with the best performance, measured by balanced accuracy.
Q-Check was enrolled parallelly on HF cows and GS herds between DIM 5 and 50. The data were derived from weekly collected milk and blood samples analysed with FTIR spectroscopy and additional fatty acids panels. This data set built the basis to develop a prediction model according to the rtFMS approach, described by TREMBLAY et al. (2019). Our project partners from the School of Veterinary Science in Wisconsin provided the computing and deep learning sources and realized the calculations for the complex prediction model.

4.1. Development of the prediction models in eight steps

To utilize the rtFMS method according to TREMBLAY et al. (2019), eight main steps towards the final model as presented in Figure 2 are required. First, all data need to be cleaned and prepared for the processing. The second step is the definition of outcome values. To predict HYK and PMAS, the outcome values were determined to be blood BHB ≥ 1.2 mmol/L and blood NEFA ≥ 0.7 mmol/L.

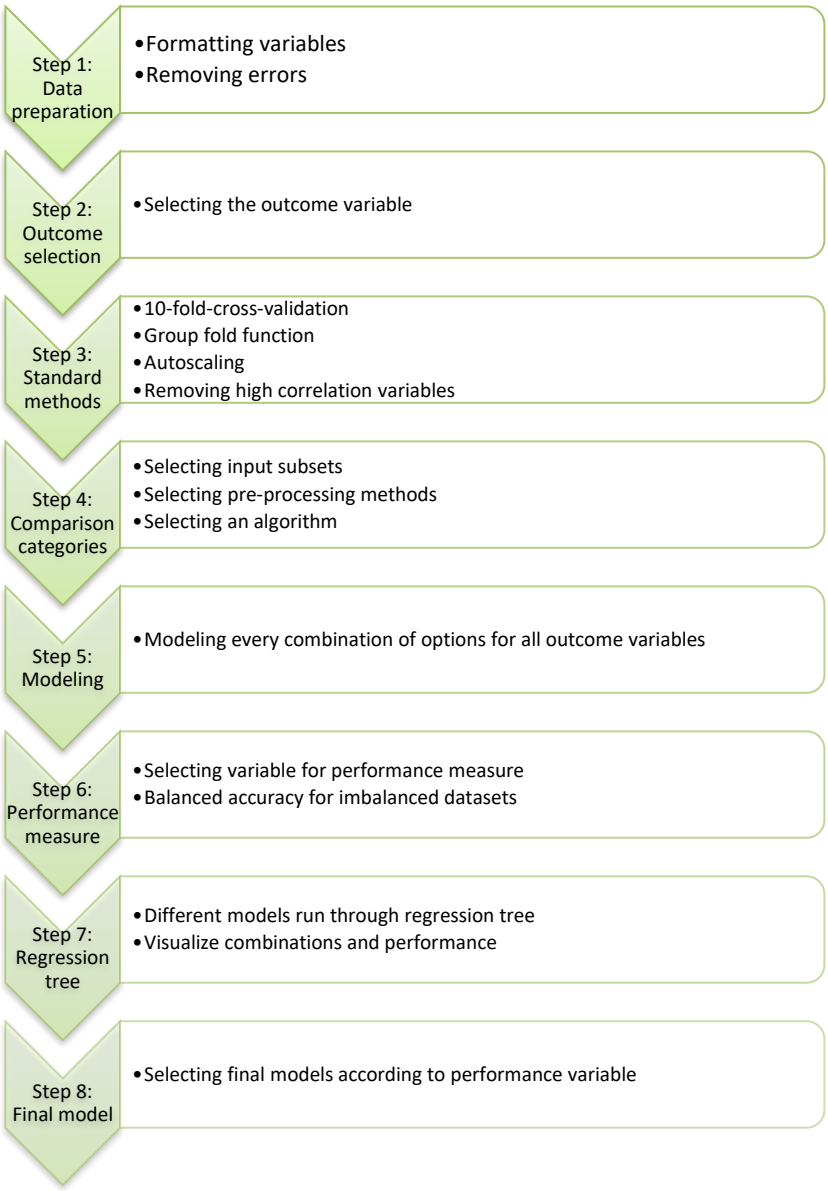

Figure 2 Process scheme for modeling a predictive model with regression tree full model selection (rtFMS) in eight steps based on TREMBLAY et al. (2019)

In step three, standard methods for data processing were applied. Step four included the decision for different options in three categories, input subset, pre-processing, and algorithm. The combination of the different modeling options, Steps 5 and 6, resulted in 329 models for the outcome value of NEFA and 669 model combinations for the outcome value of BHB. To compare the performance of the different models, the regression tree was utilized in step 7. The performance measure is balanced accuracy, which is recommended for imbalanced data sets. The model with the best performance in the final decision node was chosen in step 8 (TREMBLAY et al., 2019; KUHN AND JOHNSON, 2013; JAPKOWICZ AND STEPHEN, 2002; HOTHORN AND ZEILEIS, 2015). The regression trees are presented in the appendices 5 and 6. Two final models are defined for both outcome variables, BHB#1 and BHB#2 as well as NEFA#1 and NEFA#2.

4.2. Impact of model variables on the prediction

Additionally, all finally chosen input variables were examined through a correlogram using the Pearson`s correlation coefficient. Assuming a normal distribution of two variables, the calculation of the Pearson's correlation coefficient quantifies the strength of their relationship. It takes values between -1, inverse correlation, and +1, strong correlation. If no correlation is present, the Pearson's coefficient is zero. Values of more than 0.7 are regarded as strong correlation. All input variables with a correlation coefficient ≥ 0.7 are listed in the predictors ranking (KUHN AND JOHNSON, 2013; HAZRA AND GOGTAY, 2016)

Variables of final models, BHB#1, BHB#2, NEFA#1, and NEFA#2 are scaled by the Pearson's correlation coefficient analysis, most associated variable is scaled with 100, others are following in relation to it. The table presents the four predictors with highest correlation to the prediction performance.

Table 1 Ranking of the important predictors for the final prediction models

		BHB#1	BHB#2	NEFA#1	NEFA#2
Predictor 1	Variable	Wavenumber 1,229.745 cm^{-1}	Fatty Acids C21:1	Fatty Acids PC5 (C20:3, C22:6, C30:1)	Fatty Acids C24:5
	Scale	100	100	100	100
Predictor 2	Variable	Wavenumber 2,972.209 cm^{-1}	Fatty Acids C13:0	Fatty Acids PC1	Fatty Acids C30:1
	Scale	49.21	90.04	71.51	91.50
Predictor 3	Variable	Wavenumber 1,283.715 cm^{-1}	Fatty Acids C14:1	Fatty Acids PC6	Heifer_cow
	Scale	48.09	67.39	25.46	57.88
Predictor 4	Variable	Heifer_cow	Heifer_cow	Fatty Acids PC4	Fatty Acids C22_5
	Scale	47.24	10.86	0	48.90

BHB = ß-hydroxybutyrate

NEFA = nonesterified fatty acids

The variables with the most significant correlation according to Pearson's coefficient was scaled to 100. The remaining variables were ranked from 100 to 0 according to their correlation with the model performance. This ranking makes the influence transparent and supports the validation and evaluation of different input

variables. The effect of the number of parities for example was evaluated within the predictors ranking as presented in the Table 2 with the term "HeiferCowHeifer". The x-numbers, infrared wavenumbers, are presenting areas in the IR spectra, with impact on the prediction. The absorption bands with the identified wavenumbers of importance are presented in the appendix 7.

Table 2 Ranking of variables according to their impact on the prediction presented by the example of final model BHB#1, scaled in descending order of their impact from 100 to 0

Variables	**Scale**
X1229.745	100
X2972.205	49.21338
X1283.715	48.08956
HeiferCowHeifer	47.24482
X1572.84	46.88523
X1322.265	40.35804
X1387.8	34.77131
X2976.06	34.60437
X1703.91	24.74723
X1318.41	17.84739
X1449.48	17.64743
X1584.405	17.19656
X2964.495	16.436
X3006.9	15.82632
X2035.44	9.607511
X952.185	9.178496
X1861.965	6.091212
X2382.39	5.458686
X1148.79	4.338566
MILK_YIELD	4.142006
X1225.89	2.611003
X1757.88	2.312663
X1117.95	2.07487
X2305.29	1.435998
X998.445	1.259756
DIM	0.262297
X2189.64	0.235865

x- numbers = Infrared-wavenumbers

heifercowheifer = variable of being heifer or cow

MILK_YIELD = variable of milk production

DIM = variable of day in milk

4.3. Evaluation of variables and preconditions of the prediction models

To identify the best day in milk for sampling and the optimal sampling frequency for a routine milk testing based on this, the prevalence risk ratios per lactation week were calculated. It is defined for a positive prediction as:

(true positive predicted / total positive predicted) / (false negative predicted / total negative predicted)

The optimal frequency as the interval of sampling when most of the elevated cows could be detected and show the highest prevalence was determined subsequently. It was assumed that cows with increased values, which occurred in weeks with lower prevalence, are also a subset of the cows showing increased values in all weeks with higher prevalence. The hit rate $\boldsymbol{h}$ is the ratio of the prevalence in the measurement interval $\boldsymbol{p_i}$ in relation to the prevalence in the interval with the highest value $\boldsymbol{p_{max}}$:

$$\boldsymbol{h = \frac{p_i}{p_{max}}}$$

The missing rate $\boldsymbol{m}$ is the part of affected cows which are not detected because of the time passing between the milk samples:

$$\boldsymbol{m = 1 - h}$$

The evaluation of the effect of the daytime of sampling was considered within the predictors ranking of the input variable “AM/PM-effect”. The samples were marked with a timestamp and the different daytimes were compared with the prediction performance.

The impact of different breeds needs to be evaluated for the recommendation of an optimal prediction tool for a routine monitoring of HYK and PMAS in dairy cows. Therefore, the data set was breed-stratified and modelled for the two dairy breeds, HF and GS, simultaneously. The impact on the performance of the prediction models was evaluated with the statistical parameters (CI ≥ 95 %).

The variable of primi- or multiparous cows was suspected to have a high impact on the prediction. The correlation between the variable and the prediction models was ranked on position three and four, respectively, in the predictors ranking. In contrary, the calculation of the prevalence risk ratios for heifers and for multiparous cows presented only small differences in their probability to have a positive prediction.

To validate if the standardization of the data was necessary, the models were applied on a standardized and on a non-standardized data set. The OptiMIR Standardization project (GRELET et al., 2017; BARRY M. WISE, 1996) was developed to balance IR spectra from different machines and to render them comparable.

The evaluation of the different input variables targets to implement a practical application of the prediction tool. The implementation of the prediction tool has been the main objective of the Q-Check project and therefore of the present study. To provide a routine monitoring tool to most of the milk producers in Germany, the monthly MLP could be used as an infrastructure to collect routine milk samples. It already provides data of the milk composition and somatic cell count for each cow analysed by FTIR spectroscopy. A percentage of 88 % of the German dairy farms take part in the systematic MLP of the federal milk control associations (DEUTSCHER VERBAND FÜR LEISTUNGS- UND QUALITÄTSPRÜFUNGEN E.V., 2020). Our study aimed to identify the preconditions required for the implementation of routine herd health monitoring that targets the identification of metabolic imbalances and to provide an effective predicting of cows at risk for HYK and PMAS.

IV. PUBLICATIONS

1. Herd health monitoring in dairy farms – discover metabolic diseases. An overview

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Review Article

Herd health monitoring in dairy farms – discover metabolic diseases

AN OVERVIEW

Gesundheitsmonitoring in Milchviehherden – Stoffwechselstörungen rechtzeitig erkennen. Ein Überblick

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Schlüsselwörter

Ketose, Produktionskrankheiten, Stoffwechsel, Diagnostik, Fourier-Transform-Infrarotspektroskopie, Herdengesundheit

Key words

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ZUSAMMENFASSUNG

Die Transitperiode, 3 Wochen vor bis 3 Wochen nach der Kalbung, ist unvermeidbar mit einer Phase negativer Energiebilanz verbunden, die Stoffwechselstörungen wie beispielsweise Ketose verursachen kann. Die subklinische Ketose (SCK) wird definiert als Erhöhung der Konzentration an β-Hydroxybutyrat (BHB) im Blut auf mehr als 1,2 mmol/l. Der Grenzwert für die Konzentration nicht veresterter freier Fettsäuren (NEFA) im Blut liegt bei 0,7 mmol/l. Ab diesem Wert ist, laut einer aktuellen Studie, die Entwicklung einer Adaptationsstörung des Stoffwechsels (Poor Metabolic Adaptation Syndrome, PMAS) wahrscheinlich. Mit einer Herdenprävalenz von 21% und einer Inzidenz von annähernd 40% in den ersten beiden Laktationswochen stellt die SCK ein bedeutendes Problem der Herdengesundheit dar. Die Milchleistung sinkt bei jeder ketotischen Kuh in den ersten beiden Wochen nach der Kalbung um 3–5,3 kg pro Tag, über die gesamte Laktationsperiode von 305 Tagen durchschnittlich um 112 kg (SD 89 kg). Obwohl die Kuh in diesem Stadium keine klinischen Anzeichen einer Ketose zeigt, besteht ein erhöhtes Risiko für die Entwicklung weiterer Produktionskrankheiten (z. B. Nachgeburtsverhaltung, Metritis, Labmagenverlagerung, Lahmheiten und klinische Ketose) und in der Folge ist mit einer verminderten Milchleistung zu rechnen. Die Herdengesundheit verschlechtert sich und das Risiko für vorzeitige Abgänge nimmt zu. Der finanzielle Aspekt ist für die Betriebe ebenso relevant. Dieser setzt sich aus den Kosten für vorzeitige Abgänge, reduzierte Milchleistung, Reproduktionsstörungen und assoziierte Produktionskrankheiten zusammen. Die Kosten pro SCK-Fall werden in der Literatur unterschiedlich kalkuliert und liegen zwischen 78 $ und 289 $. Der Goldstandard zur Diagnose der SCK ist die fotometrische Messung von BHB im Blut. Diese Methode ist zwar exakt, aber durch die Analyse im Labor verzögert sich das Ergebnis. Es gibt einige direkte Schnelltestverfahren, wie Harn- oder Milchteststreifen, mit denen sich eine erhöhte Ketonkörperkonzentration schnell feststellen lässt. Aufgrund des hohen Anteils falsch negativer Ergebnisse und des erforderlichen erheblichen Arbeitsaufwands bei der Probengewinnung eignen sich diese Tests nicht für ein Herdenmonitoring. Für ein Gesundheitsmonitoring des Bestands kommt die Fourier-Transform-Infrarotspektroskopie (FTIR-Spektroskopie) in Betracht, die in der Milchleistungsprüfung zur Bestimmung der Milchinhaltsstoffe eingesetzt wird. Sie ermöglicht eine kostengünstige und rasche Bestimmung der BHB- und NEFA-Konzentration in der Milch mit einer Spezifität von 83,8% und einer Sensitivität von 82,4%. Zusammenfassend ist festzuhalten, dass sich mit einer rechtzeitigen und einfachen Identifizierung von Kühen mit subklinischen Stoffwechselstörungen finanzielle Einbußen und Verluste in der Milchleistung reduzieren lassen.

ABSTRACT

The transition period, 3 weeks before and 3 weeks after calving, is inevitably accompanied by a negative energy balance, which sometimes causes metabolic disturbances, such as ketosis. Subclinical ketosis (SCK) is defined as an increase in the β-hydroxybutyrate (BHB) concentration to ≥ 1.2 mmol/l in the blood. According to a recent study, a value of ≥ 0.7 mmol/l of non-esterified fatty

acids (NEFA) in the blood indicates the potential development of the poor metabolic adaption syndrome (PMAS). With a herd prevalence of 21%, and an incidence of approximately 40% within the first 2 weeks after calving, SCK is a relevant herd health problem. The milk yield decreases in the first 2 weeks postpartum by 3–5.3 kg/d for each ketotic cow, and the total milk reduction through the whole lactation period of 305 days averages 112 kg (SD 89 kg). Although the cow does not display any clinical signs of ketosis at this stage, the risk of developing associated production diseases like retained placenta, metritis, displaced abomasum, lameness and clinical ketosis increases and the expected performance in terms of milk production will decrease. The herd health status deteriorates and the risk for early culling increases. Another impact factor is the financial aspect, which includes costs for early death, reduced milk production, reproduction losses, and associated production diseases. In the literature, the calculated costs per SCK case vary between $°78 and $°289 The gold standard diagnostic test for SCK is the photometric measurement of BHB in blood. This method is accurate, but results are delayed due to the required laboratory analysis. There are also some rapid cow-side tests, i. e. urine or milk strip tests available to identify ketotic cows. The common disadvantage of these methods is that they are not suitable for herd health monitoring because of the need to collect samples from each cow manually and the high rates of false negative results. However, Fourier transform infrared spectroscopy is suitable for herd health monitoring. It is already being used for the analysis of milk composition. This inexpensive, rapid and simple technique has a specificity of 83.8% and a sensitivity of 82.4%. Therefore, FTIR is an early and easy method for detecting ketotic cows, that could help reduce financial and performance losses associated with ketosis.

INTRODUCTION

The peripartal timeis a critical time for dairy cows. The transition period,lasts from 3 weeks antepartum until 3 weeks postpartum. Within this time, the cows need to change from anabolic to catabolic metabolism (38).

Some cows cope well with the catabolic situation, some develop metabolic disorders like subclinical ketosis (SCK) and clinical ketosis. In the last few decades, the milk yield of dairy cows was increased by selective breeding. Cows with high performance in milk production, require improved feeding and health management. The demand for energy intake during the beginning of lactation rises from 30% to 50% as compared to the antepartum energy intake (3), (28). After calving, the dry matter intake is reduced. The gap between the nutrient demand and the available fuel cause a negative energy balance (NEB). To adapt to NEB and to provide the necessary energy, adipose tissue is mobilized and ketone bodies are produced. Due to the hormonal situation during the transition period, the available energy is used to ensure milk production (2). In some cases, the animal is not able to cope with NEB, which leads to exaggerated ketogenesis and fat mobilisation (28). The mobilized substances are transported to the liver, but the capacity of the liver cells to metabolize ketone bodies and fat is limited. If the fat mobilisation exceeds this capacity, the liver begins to store the fat in its cells and they lose their functionality (13). The high values of triglycerides, non-esterified fatty acids (NEFA), and ketone bodies are responsible for inappetence and a reduced feed intake, which further aggravates the situation (2). This leads to susceptibility to infection, reduced gut mobility and local circulatory disturbance (35). The challenge of adapting to the NEB is associated with an increased incidence of many production diseases, such as displaced abomasum, milk fever, reproductive problems, mastitis, subclinical and clinical ketosis (38).

Recent studies revealed a herd prevalence of SCK of 21.8% in Europe with a level of β-hydroxybutyrate (BHB) ≥ 1.2 mmol/l being defined as hyperketonaemia without clinical signs (46). Clinical ketosis is defined as elevated BHB concentration and clinical signs. Oetzel et al. (37) and Mc Art et al. (33) defined BHB values of ≥ 2.9 mmol/l BHB in blood as clinical ketosis. Nonetheless, there are documented cases with BHB concentrations higher than 3.0 mmol/l without clinical signs (37). Clinical signs are described as reduction in milk production, feed intake, foregut motility, massive loss of bodyweight, dry and dark faeces, unwillingness to move, and even paresis. Neurologic signs like aggressiveness, nervousness, trembling, and roaring can also be observed (2), (4), (13).

Recent studies revealed that the influence of elevated blood NEFA values was underestimated. Tremblay et al. (48) describede that elevated BHB values do not necessarily correspond with indicators of the poor metabolic adaption syndrome (PMAS) such as decreased milk production, decreased rumen fill, reduced rumen contractions or elevated liver enzymes. On the contrary, elevated blood NEFA values were found to be significantly associated with the PMAS indicators (48). Ketogenesis is a physiological process and ketone bodies are an important fuel for the organism. The transition to pathologic ketosis depends only on the ability to adapt to the NEB.

The problems described above are widespread in dairy farms and lead to reduced herd health and to negative financial impacts. The objective of this overview is to underscore the necessity of routine herd monitoring strategies to detect metabolic disorders at an early stage. These strategies need to be practicable for farmers and their consulting veterinarians in their daily work. They should provide proven reliability and accuracy and improve the health status of dairy herds.

Although, the topic of “ketosis” has been examined for many years, recent research targets automatically screening and routine monitoring to avoid both, economic loss and the clinical disease itself. This article emphasizes the significance of ketosis as a herd problem that should be approached by preventive and not reactive methods.

LITERATURE SEARCH

This paper is based on research in subject related literature. For this research several online platforms were used (Table 1). Most articles were found on Pubmed, Web of Science, Google Scholar, and in the online catalogue of the university library of the Ludwigs-Maximilian-University Munich. Quotes also were collected from official websites of the European Food Safety Authority, the "Deutscher Verband für Leistungs- und Qualitätsprüfungen e. V." (DLQ) and of the German Federal Statistical Office.

Table 1 Online services used for literature research.

Tab. 1 Für die Literatursuche verwendete Onlinedienste.

Name	Website
Pubmed	https://www.ncbi.nlm.nih.gov/pubmed/
Web of Science	http://wokinfo.com/
Google Scholar	https://scholar.google.de/
Online catalogue of the university library	https://opac.ub.uni-muenchen.de/TouchPoint/start.do?View=sunrise&Language=de&Branch=0
European Food Safety Authority	http://www.efsa.europa.eu/de
German Federal Statistical Office	https://www.destatis.de/DE/Startseite.html

The search and screening for papers to reveal the impact of ketosis in dairy farms was conducted with the key words e. g. "ketosis", "financial impact", "production diseases", "metabolism", "prevalence", "meta-analysis", and "review", in different combinations. The second topic was the description of implemented diagnostic strategies and tools. The keywords were e. g. "ketosis diagnosis", "cow-side test", "diagnosis", "test kit", "blood test", "meta-analysis", and "review".

Further research in the literature revealed an appropriate technique to monitor the milk composition, the Fourier transform infrared spectroscopy. To properly describe this technology, chemistry literature was consulted. Two textbooks were quoted in which the Fourier transform spectroscopy is described. The key words were e. g. "FTIR-spectroscopy", "Fourier transform", "spectroscopy", "spectrometry", and "introduction". The next step was the examination of FTIR-spectroscopy as part of the routine milk analysis. Literature about the technical aspects of IR-spectroscopy as well as on routine milk analysis was also reviewed. The search was based on key words like "FTIR-spectroscopy", "monitoring", "routine monitoring", "screening", and "ketosis".

The resulting data sets were evaluated on the basis of different aspects. One rating criterion was the kind of paper. Meta-analyses were rated highest, followed by studies with representative scope of examined cases, and finally reviews were consulted. Another criterion was the impact factor of the publishing magazine. A few sources were not peer-reviewed, such as conference presentations, the EFSA Journal (European Food Safety Association), the website of the DLQ and the reports of the German Federal Statistical Office. One presentation by Todd Duffield from 2011 was included. Duffield is author of many papers that, based on

the aforementioned rating criteria, were ranked highly. The quoted source is from a presentation of the Canadian Veterinary Medical Association at the World and Trade Convention in 2011. In view of the high reputation of the author of the presentation, as well as of the EFSA, the DLQ and the German Federal Statistical Office as institutions, the sources were considered as reliable.

The current topic of ketosis and related themes are widespread in subject specific literature. The main challenge is to select qualified and reliable sources. As only a quality and not a quantity search was performed, this overview cannot be considered as a meta-analysis. Altogether, 41 papers were examined, however, in the end, 37 papers and 3 textbook chapters, 4 journals from authorities, and 3 online presentations formed the basis of this overview.

RESULTS OF THE LITERATURE SEATCH

The reviewed literature revealed strong arguments that support herd monitoring. The different impact factors of SCK are described in many studies. The main facts are summarized in this section.

Change in the structure of dairy farms

Considering the change in the structure of dairy farms, herd monitoring is a fundamental requirement for herd management. Although the number of dairy farms in Germany decreased by 2.7% between November 2016 and May 2017, the amount of dairy cattle was reduced by only 0.1%. In absolute figures, 1855 farms shut down but the total amount of dairy cows was only reduced by 3351 cows. The structure of dairy farms is changing, and herd size has also changed over the last decades. While the number of herds with over 100 cows is rising, there are fewer herds with less than 100 cows. The average German milk price in May 2017 was 33.37 cents per kilogram, with a daily production on average of 21 kg per cow and a performance of all German dairy cows of 2670000 t. If the milk production of a herd decreases, the milk quantity delivered by the farmer to the dairy factories will decrease as well. These figures show that a reduced milk yield caused by ketosis is a real damage to farmers' daily income (5), (6), (7).

Prevalence and incidence of ketosis

The results of the following studies lead to the conclusion that ketosis, whether of the subclinical (SCK) or clinical (CK) form, is a herd problem in dairy herds and not a single animal disease. Five studies conducted during the last few years were examined to answer the question of epidemiology. The results of all of these studies showed a significant occurrence of ketosis in dairy herds (Table 2).

Table 2 Summary of herd prevalence and incidence figures of ketosis within the quoted literature.

Tab. 2 Zusammenfassung der Herdenprävalenz und -inzidenz der Ketose in der zitierten Literatur.

Author	Year	Kind of ketosis	Mean herd prevalence	Mean herd incidence
Suthar et al. (46)	2013	SCK	21.80%	
Duffield et al. (15)	2000	SCK	21%	
Duffield et al. (15)	2000	CK	8.5%	
Tatone et al. (47)	2017	SCK	21%	
Mc Art et al. (33)	2012	SCK		43%
Gordon et al. (25)	2013	SCK		43%
CK = clinical ketosis, SCK = subclinical ketosis				

In 2013, Suthar et al. (46) published a herd prevalence of SCK of 21.8% in Europe with a level of β-hydroxybutyrate (BHB) $\geq$ 1.2 mmol/l being defined as hyperketonaemia without clinical signs (46). Tatone et al. (47) quoted a within-herd prevalence of 21% in 791 Canadian herds (47). Duffield et al. (15) found a prevalence of SCK of 8–34% in the first 2 months of lactation. Compared to these rates, the prevalence of CK of 2–15% is quite low (15). The wide range of prevalence of SCK and CK may arise from the enormous changes the cows undergo within the first two months of lactation. The dynamic process from zero milk to rising amounts of milk in early lactation and later to maintaining high performance lactation changes the metabolic situation day by day. McArt et al. (33) published an incidence of SCK between days 3 and 16 of 43% (33). The incidence increases with the number of lactations from 16% for heifers to 47% for cows in their third and higher lactation. Especially multiparous cows that were hyperketonaemic in the previous lactation have an incidence of 60% of developing SCK again in the following lactation (25). The results in terms of SCK or CK occurrence reflect the scope of challenge farmers and veterinarians are faced with.

Reduction in milk yield caused by ketosis

High performance cows are more likely to become ketotic due to their high nutrient requirements. However, although their milk yield is reduced in the case of a ketosis, their production over a 305-day lactation period is similar to non-high performing cows. Miettinen et al. (34) concluded from their study that even subclinical ketotic cows do not exploit their full milk yield potential (34). If the metabolism were not out of balance, high producers, who are more likely to become ketotic, could give even more milk (27).

The cows start to reduce their milk yield 2–4 weeks before the diagnosis is made. The loss in milk production peaks at two weeks postpartum with a loss of 3–5.3 kg per day and lasts for a varying amount of time. In higher parities the reduction is more severe than in the first or second parity (40). SCK causes a decrease in milk production of 0.5 kg for each 0.1 mmol/l BHB above the threshold of 1.2 mmol/l. Cows, with SCK are 3.0 times more likely (relative risk, 95% confidence interval = 2.2–4.2, $p < 0.001$) to be culled or die than non-ketotic

cows (33). A meta-analysis regarding the impact of SCK on cattle revealed a loss in milk production with an average of 112 kg (SD 89 kg) within a 305-day lactation period (39).

Ketosis associated production diseases and impacts on animal health

Another key factor of SCK is the association with other production diseases and reproductive problems (15). Mulligan and Doherty et al. (36) underscore the impact of production diseases and their association among them. The unavoidable NEB and the poor status of the immune system after calving are often followed by ketosis, mastitis, retained placenta, displaced abomasum, and other production diseases (36). Esposito et al. (18) revealed a significant influence of the metabolic status on the immune system. The poor immune status around calving is caused and supported by the protein and energy malnutrition, which lead to a NEB (9). Additionally, the hormonal change within the transition period are also responsible for the vulnerability of the cows. After calving the insulin, leptin and gestagen values decrease and the oestrogen, glucocorticoid and growth hormone values increase and lead to rapid mobilization of adipose tissue and support the metabolic stress in the organism. (18).

Suthar et al. (46) examined the association between hyperketonaemia and the prevalent production diseases like metritis, mastitis, displaced abomasum, lameness, and clinical ketosis in 5884 cows. Blood BHB values were obtained between 2–15 days in milk (DIM). They found different BHB thresholds for SCK diagnosis, above which the occurrence of the several diseases could be predicted with high accuracy. They also calculated the risk for a cow to develop one of those diseases by defining the odds ratio (OR). Although Suthar et al. (46) identified different optimum thresholds, the difference in OR was not significant. Therefore, they recommend a uniform threshold of 1.2 mmol/l BHB for SCK diagnosis and to predict the risk of developing a production disease.

The analysis of 5037 cows showed OR of 1.7 for metritis with a blood BHB $\geq$ 1.4 mmol/l. In 5023 cows the OR for CK was 10.5 for the BHB threshold $\geq$ 1.1 mmol/l. Lameness occurs with OR of 1.8 when blood BHB is $\geq$ 1.1 mmol/l, as examined in 5041 cows. For the risk of displaced abomasum, 5412 cows were examined, and the OR was 6.9 with a threshold of $\geq$ 1.7 mmol/l blood BHB (46) (Table 3).

Table 3 Comparison of the odds ratios to develop production diseases associated with elevated levels of β-hydroxybutyrate (BHB) (46).

Tab. 3 Vergleich der Wahrscheinlichkeiten eine der Produktionskrankheiten zu entwickeln, die mit erhöhter β-Hydroxybutyrat-Konzentration (BHB) assoziiert sind (46).

Disease	Total no. of cows	BHB threshold (mmol/l)	Cows above cut point (n)	Risk measure							
				+PV	–PV	OR	95% CI	p-value	Se	Sp	LR+
Metritis	5037	≥ 1.4	162	15.9	91.8	2.1	1.8–2.6	> 0.0001	28.5	84.1	1.8
Clinical ketosis	5023	≥ 1,1	179	12.0	99.1	14.0	10.4–20.7	> 0.0001	81.2	77.1	3.6
Displaced abomasum	5412	≥ 1,7	98	11.7	98.8	10.7	7.6–14.9	> 0.0001	60.4	87.4	4.8
Lameness	5041	≥ 1,1	77	5.1	97.3	2.1	1.5–2.7	> 0.0001	39.1	75.4	1.6
+PV = positive predictive value, –PV = negative predictive value, OR = odds ratio, CI = confidence interval, p-value = probability value, Se = sensitivity, Sp = specificity, LR+ = positive likelihood ratio											

Raboisson et al. (39) summarized in a meta-analysis the association between SCK and production diseases. This study reflects the great heterogeneity of this topic in the literature. They compared 10 statistical models of 3 publications. Finally, they proved a risk for left displaced abomasum with an OR of 3.3 and an OR of 5.38 for clinical ketosis in cows, which already have hyperketonaemia. The OR of culling within 60 days after calving is retained with 1.92. The OR for metritis associated with SCK is 1.94. For retained placenta the OR is 1.52. The OR for lameness in cows suffering from SCK is 2.0. The impact of SCK on reproductive performance was not significant due to a high heterogeneity of variables in the studies (39) (Table 4).

Table 4 Comparison of the odds ratios to develop production diseases associated with elevated levels of β-hydroxybutyrate (BHB) (39).

Tab. 4 Vergleich der Wahrscheinlichkeiten eine der Produktionskrankheiten zu entwickeln, die mit erhöhter β-Hydroxybutyrat-Konzentration (BHB) assoziiert sind (39).

Disease	OR	95% CI	p-value	Number of statistical models/publications
Displaced abomasum	3.30	2.60-4.25	< 0.001	10/3
Clinical ketosis	5.38	3.27-8.38	< 0.001	10/3
Culling within 60 days p. p.	1.92	1.60-2.30	< 0.001	10/3
Metritis	1.94	1.75-2.10	< 0.001	10/3
Lameness	2.0			10/3
OR = odds ratio, CI = confidence interval, p-value = probability value				

A meta-analysis about the association between hyperketonaemia, high levels of NEFA, and production diseases revealed a combined risk for metritis of 1.912. For retained placenta the value amounts to 1.51 (1) (Table 5).

Table 5 Comparison of the odds ratios to develop production diseases associated with elevated levels of β-hydroxybutyrate (BHB) (1).

Tab. 5 Vergleich der Wahrscheinlichkeiten eine der Produktionskrankheiten zu entwickeln, die mit erhöhter β-Hydroxybutyrat-Konzentration (BHB) assoziiert sind (1).

Disease	OR	95% CI	p-value	Number of statistical models/publications
Placental retention	1.51	1.19-1.92	< 0.0006	3/3
Metritis	1.912	1.70-2.15	< 0.0001	11/6
OR = odds ratio, CI = confidence interval, p-value = probability value				

As far as Scott et al. (44) are concerned, the terms “relative risk” and “odds ratio” are interchangeable in clinical trials and can be used equally in the description of risk.

Financial impact of ketosis

The main reason for farmers to implement a herd health monitoring program is the financial impact of production diseases in a dairy herd (16). Gohary et al. (24) calculated the costs of each case of SCK to be $ 203, which accounts for culling and death (13%), milk production losses (22%), reproduction losses (28%), and developing other diseases (37%) (24). This study shows, it is not the veterinary treatment that is responsible for the negative financial impact of SCK but it is the directly associated consequences that are (24). Geishauser et al. (22) published average costs of $ 78 per case of SCK. They underscore the high financial impact on herds due to an assumed prevalence of SCK up to 40% (22) (Table 6). McArt et al. (32) developed a deterministic economic model to calculate the component costs for a case of hyperketonaemia. The main components of their cost analysis were the losses in reproduction (34%), losses by death (26%), and losses in future milk yield (26%). On average, in primi- and multiparous cows, each case of hyperketonaemia is priced at $ 289 (32). Assuming a prevalence of 21.8% in a herd with 100 cows (46), the financial impact varies from $ 2550 to $ 4425 in total for the transition periods of the whole herd, depending on the source used for the calculated costs (24), (32) (Fig. 1). The calculation of expenses per case is very complex. Therefore, Raboisson et al. (38) described several models. The mean total costs per case for cows with SCK in an average economic system is calculated at 257 €. They also describe different alternative calculation models that consider prevalence, feed margin or milk production, which decreases or increases the costs per case (38).

Table 6 Economic impact per case of subclinical ketosis (SCK).

Tab. 6 Finanzielle Einbußen pro Fall von subklinischer Ketose (SCK).

Author	Year	Mean costs per SCK case	Mean herd prevalence of SCK
Gohary et al. (24)	2016	$ 203	21%
Raboisson et al. (38)	2015	$ 285 (257 €*)	40%
McArt et al. (32)	2015	$ 289	31.5%
Geishauser et al. (22)	2001	$ 78	39.5%
* Yearly average exchange rate of 2015			

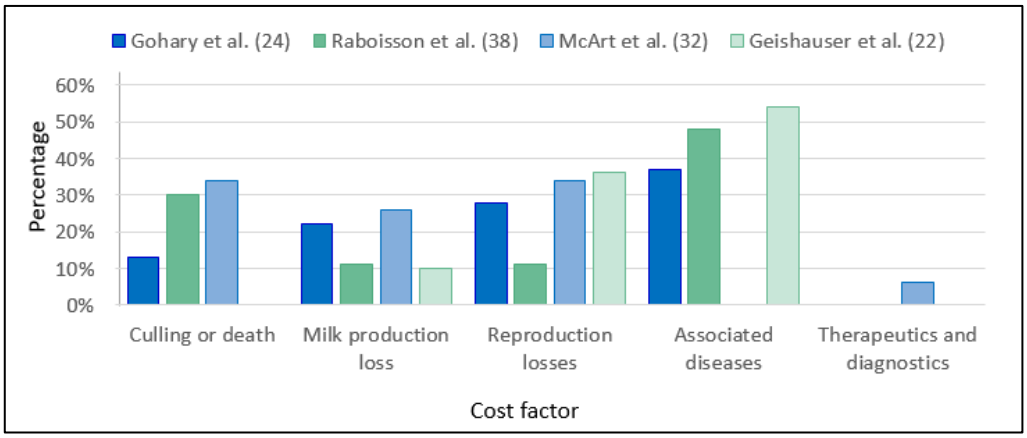

Fig. 1 Composition of costs per case of subclinical ketosis. © S. Gruber.

Abb. 1 Zusammensetzung der Kosten je Fall von subklinischer Ketose. © S. Gruber.

Diagnostic and monitoring methods for ketosis

In view of the arguments listed before and the impact of ketosis on modern dairy herds, it is important to examine the common diagnostic tools in herd health monitoring and early detection of ketosis for their relevance.

Body condition score and back fat thickness monitoring

A study of Busato et al. (8) revealed an association between high values of body condition score (BCS) (17) > 3.25 antepartum and a great loss of body mass in the first weeks after calving. These animals tend to have high rates of fat mobilisation which leads to increased NEFA and BHB values in their blood (8). These results are confirmed by Gillund et al. (23) and Roche et al. (41). Therefore, the monitoring of the body condition of the cows before calving is an appropriate tool for herd health management (23), (41). The farmers are able to control their herds using the BCS chart of Edmondson (17). If every cow is registered and scored once, they can be checked for changes in their BCS regularly. Gillund et al. (23) recommend for Norwegian dairy cows a BCS < 3.5 points for calving, to prevent massive fat mobilisation.

The method of measuring back fat thickness (BFT) via ultrasound requires veterinary help and is work intensive but the results are more objective and precise. The method of Staufenbiel et al. (45) is recommended to gain comparable results regarding the body condition within a herd. The subcutaneous BFT is measured in a horizontal line between tuber ischiadicum and tuber coxae, in the caudal fifth to fourth part, vertically in the junction of the os sacrum to the first caudal vertebra. The appreciable values are measured from the skin to the fascia trunci profunda, which presents itself as a white, hyperechogenic line, after subtracting 5–6 mm for the dermis (45).

Even if these methods are practicable and easy, the previously mentioned trend towards bigger herds is not helping to encourage farmers for routine monitoring due to the time and personnel effort that is needed for those methods.

Cow-side ketosis tests

Up to now the common method for detecting ketosis has been analysing milk, urine or blood of suspicious cows. However, only the minority of subclinical ketotic cows is discovered with this approach because many cows will not show symptoms and therefore will not be tested.

The diagnostic gold standard is the photometric examination of blood and the evidence of at least 1.2–1.4 mmol/l BHB (37), however other serum metabolites are also important. More than 0.5 µmol/l NEFA in the blood is evidence of a disturbance in fat metabolism (30). First of all, Oetzel et al. (37) recommended blood testing on NEFA antepartum and postpartum. With increasing rates of fat mobilisation, NEFA are rising as well. A concentration > 0.4 µmol/l indicates a decrease in feed intake, an increased NEB, and a higher risk for ketosis (37). Latest studies have confirmed, that blood NEFA values taken postpartum, DIM 5–50, are reliable indicators for the PMAS. The separation values were selected at $\leq$ 0.39 mmol/l for low PMAS observations and $\geq$ 0.7 mmol/l for high PMAS observations (48).

The blood testing method is reserved for veterinarians and has a delayed result due to the necessary examination in a laboratory. Therefore, the industry has developed many alternative cow-side tests that could be used by the farmers and show the results in real-time.

A common test is the semiquantitative KetoTest™, also called KetoLac® BHB, or Sanketopaper® (Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, JP). The herd prevalence measured with a threshold > 200 µmol/l BHB is similar to the herd prevalence measured with blood tests (37). Carrier et al. (10) compared 3 cow-side tests. KetoStix® (Bayer Corporation, Elkhart, IN, USA) detects acetoacetate in urine samples, KetoCheck powder™ (Great States Animal Health, St. Joseph, MO, US) checks for acetoacetate in milk, and the abovementioned KetoTest™ uses the detection of BHB in milk. In conclusion, these 3 tests are useful for examining single animals but they need to be used cautiously for ketosis screening in herds due to their high rates of false negative results in herds with a low prevalence of ketosis (10). Another study compared eight different products, 7 with urine samples and 1 with a milk sample. Only the KetoLac® BHB test (Hoechst, Frankfurt am Main, DE) could identify a number of ketotic cows

correctly that corresponds to the prevalence measured with blood BHB and allowed monitoring of ketosis within a herd (21).

In human medicine there are different hand-held instruments to check the blood glucose and ketone body levels. For veterinarian use there is the Precision Xtra® (Abbott Diabetes Care, Abingdon, UK) which could work with blood, milk or urine samples. Iwersen et al. (29) evaluated the results of Precision Xtra®, KetoStix® and KetoLac®. All 3 tests were validated with the gold standard method of photometric measurement of blood BHB concentration. For an SCK threshold of $\geq$ 1.4 mmol/l BHB the Precision Xtra® with blood sample had a result of 100% in specificity and sensitivity (29). Another way to use the Precicion Xtra® is to measure ketone bodies in urine and milk. With urine samples it also reaches a sensitivity of 100% but the specificity decreases to 25%. For milk samples the sensitivity was 60% and the specificity was 89% (29). Apart from good statistical results the main disadvantage is the need to handle each cow individually to gain results. The routine monitoring of ketosis in dairy cows still requires a forward-looking technology and further exploration (Table 7).

Table 7 Overview of handheld tests for detection of ketosis.

Tab. 7 Übersicht über Schnellestverfahren zum Nachweis von Ketose.

Authors	Name	Company	Measured substance	Sample fluid	Threshold	Sensitivity	Specificity
Geishauser et al. 2000 (21)	KetoLac®	Hoechst, Frankfurt am Main, DE	BHB	blood	≥ 200 µmol/l	59%	91%
Carrier et al. 2004 (10)	KetoStix®	Bayer Corporation, Elkhart, IN, USA	aceto-acetate	urine	"small cut-off"	78%	96%
	KetoStix®	Bayer Corporation, Elkhart, IN, USA	aceto-acetate	urine	"moderate cut-off"	49%	99%
	KetoCheck Powder™	Great States Animal Health, St. Joseph, MO, USA	aceto-acetate	milk	n/s	41%	99%
	KetoTest™, KetoLac®	Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, JP	BHB	milk	≥ 100 µmol/l	73%	96%
	KetoTest™, KetoLac®	Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, JP	BHB	milk	≥ 200 µmol/l	27%	99%
Iwersen et al. 2009 (29)	Precision Xtra®	Abbott Diabetes Care, Abingdon, UK	BHB	blood	≥ 1.2 mmol/l	100%	100%
	Precision Xtra®	Abbott Diabetes Care, Abingdon, UK	BHB	urine	n/s	100%	25%
	Precision Xtra®	Abbott Diabetes Care, Abingdon, UK	BHB	milk	n/s	60%	89%
van Knegsel et al. 2010 (51)	MilkoScan™ FT600	Foss Analytical A/S, Hillerød, DK	BHB	milk	≥ 1.2 mmol/l	80%	70%
	MilkoScan™ FT600	Foss Analytical A/S, Hillerød, DK	aceto-acetate	milk	n/s	80%	70%
van der Drift et al. 2012 (50)	MilkoScan™ FT600	Foss Analytical A/S, Hillerød, DK	BHB	milk	≥ 1.2 mmol/l	82.4%	83.8%
	MilkoScan™ FT600	Foss Analytical A/S, Hillerød, DK	aceto-acetate	milk	n/s	82.4%	83.8%
Santschi et al. 2016 (43)	MilkoScan™ FT6000	Foss GmbH, Hamburg, DE	BHB	milk	≥ 2.0 mmol/l	96%	89%

n/s = not stated ■

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy is another diagnostic method that is already used to determine the milk composition in the monthly milk performance tests, which are provided by the state control associations of each federal state in Germany. Almost 90% of all dairy farms in Germany participate in this process to check the quality of their milk (12).

The method is based on the difference in base rotation of each molecule. If a wavelength of infrared light has the same frequency as the base rotation of the molecule, it will absorb the energy of this wavelength and change to a higher level of rotation. The absorbed wavelength is detected in the IR-spectroscope and shows the individual spectrum of each molecule. Each molecule or substance has characteristic absorption bands in its spectra, which makes the IR-spectroscopy suitable for the identification of unknown substances. After many years of research, data-bases containing the IR-spectra of many molecules have been established to compare unknown substances with already documented substances (42). After the combination of infrared spectroscopy with the Fourier transformation, a mathematical transformation of the interference of waves, scientists have been able to analyse the whole spectra of a substance all at once. Upon comparison with the databases, the structure of the substance is quickly identified. In summary, this technique impresses because of its cheap, rapid and simple character (26). In recent times the Fourier transform infrared spectroscopy was developed and pushed further for routine monitoring in dairy cows.

Therefore, Tsenkova et al. (49) examined the FTIR-spectroscopy for its suitability as an on-line test for the determination of the milk composition over the lactation period. They determined the best accuracy for sample thicknesses of 1 mm and a wavelength in near-infrared spectroscopy of 1100–2400 nm. Tsenkova et al. (49) promoted this technology as an accurate routine test for milk composition in dairy cows throughout the whole lactation period (49). Van Knegsel et al. (51) examined the prediction of hyperketonaemia, with a reference threshold of plasma BHB $\geq$ 1.2 mmol/l, by FTIR-spectroscopy of BHB, acetoacetate, and milk fat to protein ratios in test-day milk. The results of testing milk BHB, (optimal cut-off value of 23 µmol/l), and milk acetone (optimal cut-off value of 70 µmol/l) showed higher accuracy compared with the results of testing milk fat to protein ratio. Nevertheless, the sensitivity increases up to 89% when all 3 tests, BHB, acetone and milk fat to protein ratio, were used together to detect cows with hyperketonaemia. However, due to the high rates of false positives with low prevalence, the authors recommend further studies to test the practicability of FTIR-spectroscopy in routine ketosis monitoring (51). Van der Drift et al. (50) developed a diagnostic model including FTIR-spectroscopy of BHB and acetoacetate in milk, but also parity, season, and milk fat to protein ratio to optimize the real predictive values. With this model a sensitivity of 82.4% and a specificity of 83.8% was reached. Nonetheless, the model was not considered suitable for individual cow testing due to length of test day intervals and low positive prediction values. But for herd monitoring it is considered as a practicable approach (50).

Elevated NEFA are the most significant indicator of PMAS in early lactation. However, the analysis of blood NEFA is expensive, which makes a measurement via FTIR-spectroscopy quite desirable. The application of FTIR-spectroscopy in this context allows a routine and on-line measurement of the metabolic status of the herd. It could help to alert the farmers for single animals and to control the prevalence of PMAS in the herd (48).

Santschi et al. (43) published a long-term study over 4 years with almost 500000 monthly collected samples. They tried to establish a routine FTIR-spectroscopy based on monitoring within the frame of the monthly Dairy Herd Improvement program in Canada. They used a MilkoScan™ FT6000 (Foss GmbH, Hamburg, DE) and the thresholds suggested by the company. The milk sample is negative if the value is < 0.15 mmol/l BHB. With values between 0.15 and 0.19 mmol/l BHB the sample is suspicious, with > 2.0 mmol/l BHB the sample is positive. According to the FOSS Ketosis application note 35 (20) this threshold shows 96% sensitivity and 89% specificity (20). Denis-Robichaud et al. (11) confirmed the abovementioned thresholds as the values with highest sensitivity and specificity. They compared BHB in milk with BHB in blood and published the threshold of ≥ 2.0 mmol/l BHB in milk as equal with a hyperketonaemia of ≥ 1.4mmol/l BHB in blood (11). These thresholds seem to be reliable values for classifying the cows at high or low risk for developing ketosis. The prevalence of 22.6% revealed in the study of Santschi et al. (43) is similar to former studies that used the gold standard and measured BHB in the blood.

In recent times the topic of routine monitoring with the FTIR-spectroscopy technique has become popular in Germany. A current project called “Q-Check” from the “Deutscher Verband für Leistungs- und Qualitätsprüfungen e. V.” (DLQ), “Milchprüfring Bayern e. V.” (MPR), Clinic for Ruminants of the Ludwig-Maximilian University (LMU) in Munich, and other institutions is ongoing. One working package of Q-Check under the lead of the Clinic for Ruminants (LMU) and the MPR develops a routine metabolic herd monitoring method by using FTIR-spectroscopy. In cooperation with the Department of Medical Science of the University of Wisconsin (Tremblay et al.), a prediction model for metabolic disorders should be developed. This project is still ongoing, but promising results are expected.

CONCLUSION

In view of the immense impact of SCK on dairy farms, it is important to detect ketotic cows early and easily. The European Food Safety Authority (EFSA) recommends monitoring systems for efficient prevention of production diseases and to guarantee animal welfare (19). Leblanc et al. (31) proved the advantages of preventing diseases before they become clinically relevant. Therefore, they recommend herd monitoring to check the health status of the herd (31). The chance of detecting deviations in the herd health status early increases with regular monitoring. There are different ways to approach this widespread stock problem. Until now farmers and veterinarians have often diagnosed ketosis only in suspicious cases and obviously ill animals. The results of these cow-side tests show their suitability for detecting ketotic cows. However, the main disadvantage of them is the amount of effort necessary to gain samples from the cows, which is one reason why only obviously ill animals are sampled. The non-invasive methods like body condition scoring and the measurement of backfat thickness give reliable results, but are associated with extra effort for the examination of the cows. At the moment real monitoring strategies like the FTIR-spectroscopy are rare. All studies on routine monitoring of ketosis in herds recommend the FTIR-spectroscopy as forward-thinking and an appropriate method for this subject.

The whole impact of ketosis on production including milk yield reduction, costs for recovery time, and associated diseases is the main argument for reliable and practicable ketosis monitoring. It will become necessary to develop a technical and practical solution for a routine monitoring of transition cows (14). Tremblay et al. (48) showed the significant association between elevated NEFA and severe PMAS indicators. With the application of FTIR-spectroscopy to measure milk NEFA, a prediction model for PMAS could be built. It could help the milk producers to evaluate and improve their metabolic health management (48). Applying on-line FTIR-spectroscopy in automatic milking systems may be an option in the future. In view of the complexity of this topic and the necessity to provide practicable solutions that work for the farmers and veterinarians, further research and studies are required.

Conflict of interest

The authors declare no conflict of interest.

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2. 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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Evaluation of an early warning system for elevated ß-hydroxy-butyrate and non-esterified fatty acid values based on Fourier trans- form 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 se- lection (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 Sim- mental, prediction model, herd health monitoring

Introduction

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 metabolism 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] construct- ed 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 MilkoScanTM 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. FossomaticTM 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 GlideTM 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 omit- ting 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 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].

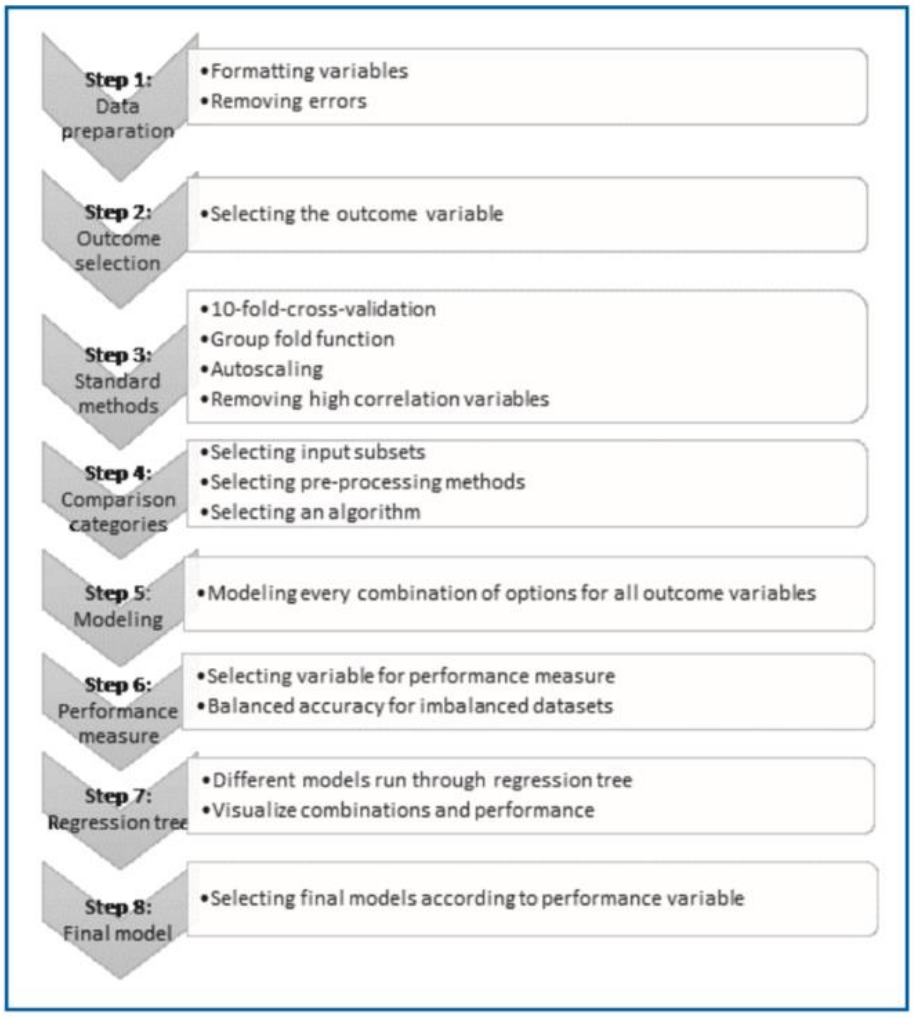

Figure 1: Process scheme for modeling a predictive model with regression tree full model selection (rtFMS) in eight steps based on Tremblay et al. (2019)

The regression tree consists of decision nodes 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 di- agnostic parameters, i.e., sensitivity, specificity, positive and negative predictive value, and likelihood ratio of a positive and a negative test.

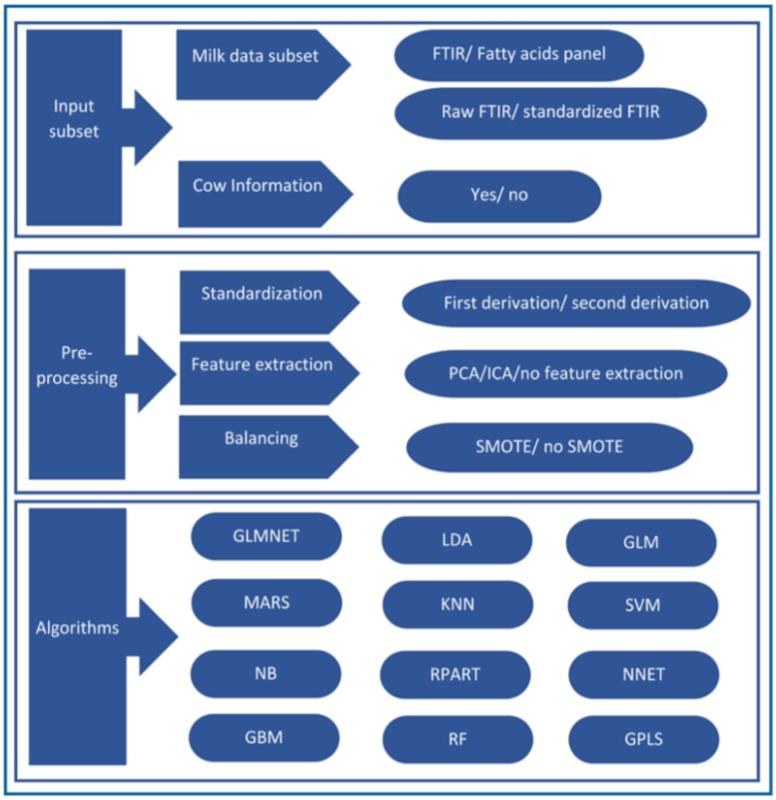

Figure 2: Models are defined as combination of one option from each category. The area (1) Input variables. The decision categories are (1.1) milk data subset with the options (1.1.1) Fouriertransform infrared spectral (FTIR) data, (1.1.2) fatty acids panels, (1.1.3) raw FTIR data, (1.1.4) standardized 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 (SMO- TE), (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)

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 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).

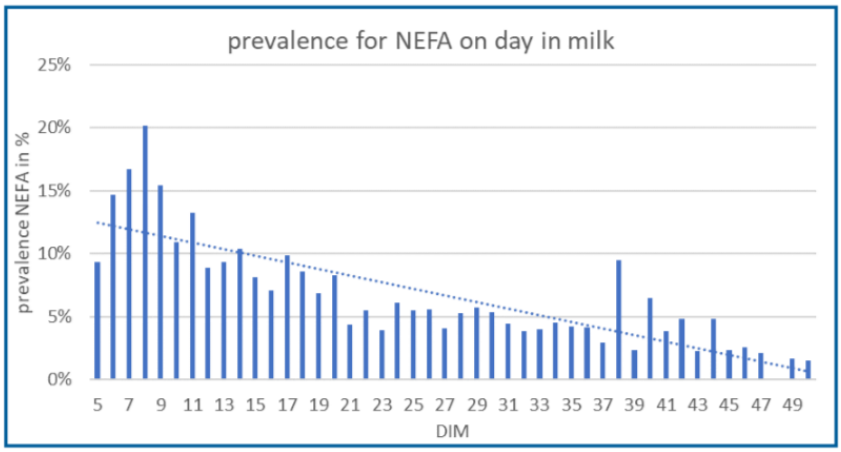

Figure 3: Proportion of samples above the cut-off values for nonesterified fatty acids (NEFAs) ≥ 0.7 mmol/l for each day in milk (DIM)

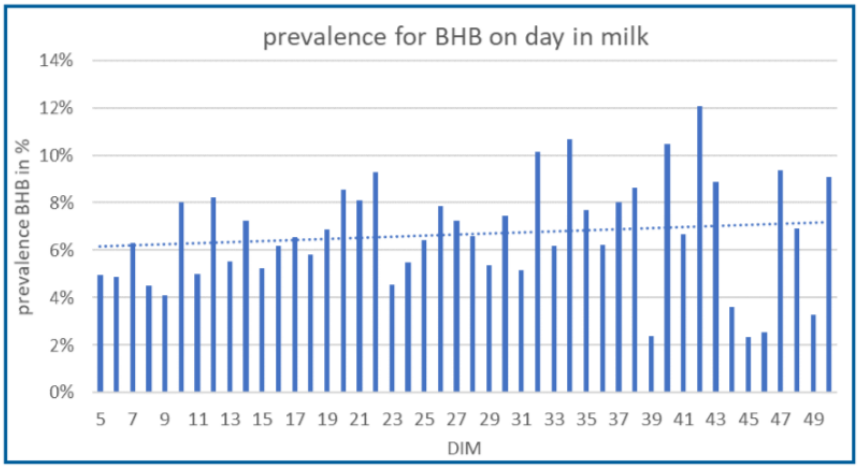

Figure 4 Proportion of samples above the cut-off values for β-hydroxybutyrate (BHB) ≥ 1.2 mmol/l for each day in milk (DIM)

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).

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**

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.

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%

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 per formance 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 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 over- lap. All models could be implemented equally. Statistical parameters and 95% CIs are reported in Table 3.

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

Discussion

Methods in data collection: We needed to fix the relevant cows for the 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 com- parison 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 de- creased 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 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 meth- od 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 im- pact 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 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 spec- ificity. 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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V. DISCUSSION

The baseline of both publications is the improvement of animal welfare through the implementation of herd health monitoring aiming at the reduction of metabolic disorders. The integrated veterinary herd health care is an indispensable part of modern dairy farming. In the first publication, the topic of herd health monitoring is discussed. A cornucopia of arguments is presented to underscore the necessity of routine monitoring of the health status in dairy herds. The second publication provides a specific solution to implement herd health monitoring on the farms. The validation of prediction models for elevated BHB and NEFA values, which are markers for metabolic disorders such as HYK and PMAS, is described. Preconditions and sampling logistics are defined and evaluated. The implementation of advanced and prospective monitoring in routine processes is prepared through this work. Both publications aim to improve animal welfare and health and present results for their practical application.

1. Projects, investigating metabolic imbalances

To investigate metabolic disorders and for their early detection, a project called FSM-Irmi[2], was set up in 2016 by the project partners Clinic for Ruminants with Ambulatory and Herd Health Services (LMU), the Dairy Herd Improvement Association of Bavaria (LKV Bayern), the Bavarian Association for raw Milk testing (MPR), and the Department of Medical Science/School of Veterinary Medicine at the University of Wisconsin. This project aimed to investigate the association between clinical symptoms caused by metabolic imbalances and the elevation of BHB and NEFA values measured in FTIR data. The collected data from milk and blood samples from cows between DIM 5 - 50 were processed by TREMBLAY et al. (2018) using principal component analysis (PCA) and cluster analysis. The results have presented a strong association between NEFA elevation and clinical symptoms. Conversely, an association between elevated BHB and clinical symptoms could not be perceived. The next step was the development of prediction models based on a modified regression tree combined with the full

[2] Frühwarnsystem für Stoffwechselerkrankungen von Milchkühen mithilfe von Infrarot-Absorptionsspektren der Milch
Development of an early warning system for subclinical ketosis by using milk infrared spectroscopy

model selection (FMS) approach to process the FTIR data collected in the above-mentioned study. This was described in TREMBLAY et al. (2019).

Simultaneously, the demand for comprehensive and transparent animal welfare and herd health monitoring in dairy farms has increased. The project Q-Check was initiated by the German Association for Dairy Herd Improvement (DLQ), funded by the BLE. The working package three (AP III), which is covered by the project partners Clinic for ruminants with Ambulatory and Herd Health Services (LMU), the LKV Bayern, the Bavarian Association for raw Milk testing (MPR), and the Department of Medical Science/School of Veterinary Medicine at the University of Wisconsin, was a subproject within Q-Check. The objective was again to collect milk and blood samples from cows in their early lactation between DIM 5 to 50 and to analyze FTIR data and corresponding blood data. In contrary to FSM-IRMI, the herds consisted of two breeds, HF and GS cows. The rtFMS approach, which had successfully been tested on the FTIR data of the FSM-IRMI project, was used to develop prediction models suitable for routine herd health monitoring. The validation and application of the prediction model described by TREMBLAY et al. (2019) has been the objective of this project and additionally, specific preconditions required for an implementation in existing milk sampling processes were determined. The validation of the rtFMS prediction tool resulted in a binary outcome regarding established cut-off values of more than 1.2 mmol/L BHB and more than 0.7 mmol/L NEFA. In ongoing investigations, a wide field for further research perspectives following the current project Q-Check has emerged. The development of the prediction models with rtFMS has been evolving towards a numeric output solution. Detailed fatty acids profiles of the milk samples were examined regarding their association with the occurrence of clinical symptoms. The investigation of the impact of Haptoglobin in the detected samples and the correlation between activity data of cows and the detected metabolites was an additional related research topic. Another result of the exploration and development of prediction models was the definition of different cow types that are clustering according to their alterations in blood and obvious symptoms. PMAS was initially defined because of the congruence between elevated NEFA values and clinical symptoms (TREMBLAY et al., 2018). Further examination of FTIR data with PCA and linear and logistic regression resulted in five types of cows, described in an unpublished manuscript by WALLESER (2020) from the School of Veterinary Medicine / University of Wisconsin. The

clustering of groups with similar metabolic characters results in a scheme of cow types that defines the metabolic profile and typical characteristics.

The “PMAS cow” is a cow that has high NEFA values but low BHB values and a high fat-to-protein ratio (FPR). Usually, a reduced milk production occurs very early during the lactation compared with unaffected cows.

The “athletic cow” is a high producing cow of higher parity which presents with high BHB values and high FPR, varying NEFA values and a milk production that equals the milk yield of unaffected animals.

The “clever cow” presents with low BHB values and a low FPR, varying NEFA values, and higher milk production than healthy cows. They reduce their milk fat to safe energy, which is the reason for their name.

The “hyperketonemic cow” has elevated BHB values, a low FPR, varying NEFA values, and a milk production comparable with the healthy group.

The “healthy cow”, used as reference group, shows low BHB and NEFA values, high FPR, and a low milk production. The low production is mainly responsible for the compensation of the NEB.

The aforementioned definition of different types of cows may improve the profiling of metabolic herd health status and yield recommendations for the specific and targeted care of these animals (WALLESER, 2020).

In summary, there is a potential research field for the improvement in animal welfare and herd health that was opened by the working package III of the Q-Check project.

2. Structural changes in dairy farming

To implement a convincing prediction tool for the German dairy farmers, it is necessary to know their needs. The size of farms in Germany has been increasing, whereas their number has been decreasing. The number of dairy farms has fallen from 91,550 in November 2010 to 59,925 in November 2019, which equals a percentage of approximately 34.55 %. However, the number of dairy cows has been declining from 4,181,679 (November 2010) to 4,011,674, (November 2019): about 4.06 % (BUNDESAMT FÜR STATISTIK, 2012, 2019). The first publication presents values from the years 2016 and 2017, which reveal a similar

trend, but the situation over a decade from 2010 to 2019 illustrates the change in agriculture more detailed. The daily work has become more digitized and automatized through several machines. Automatic milking systems (AMS), feeding or cleaning robots have been enhancing the processes for farmers. Additionally, AMSs provide a lot of production data that support the monitoring of the herd health status. It could be an explanation that larger farms are able to invest in the required technical equipment compared with smaller farms. These changes in working processes and the increasing amount of available data also make an adaptation of veterinarians and their services necessary. It could change towards a more proactive, advisory-focused supervision rather than reactive treating of animals. Additionally, there are several data sources with herd health and production data available which could be used in practical herd management. An objective of the Q-Check project was the development of an interface for these data sources and to enable their users to extract the important information. Veterinarians are to interpret the data and to derive recommendations from the available information that help to improve the herd health management.

3. HYK and PMAS

3.1. Epidemiology

In our study, we determined the thresholds of BHB values of more than 1.2 mmol/L in blood and NEFA values of more than 0.7 mmol/L blood as elevated values to detect HYK and PMAS, respectively. These values are recommended in literature as well (TREMBLAY et al., 2018; SUTHAR et al., 2013; OETZEL, 2004). SUTHAR et al. (2013) presented a prevalence of SCK, defined as 1.2 mmol/L BHB in blood, in Europe of 21,8 % and TATONE et al. (2017) of 21 % in Canadian herds. Similar results of 23 % were found in our study for German Simmental cows in Germany. However, HF cows presented a prevalence of only 10 %. One reason for this difference could be found in different herd management on the large dairy farms in Thuringia compared with smaller farms in Bavaria. The almost 2,000 HF cows are in majority from the two farms in Thuringia where the feeding and the milk production is analyzed continuously. A lack in nutritional contents in feed could be detected and solved immediately.

Another reason is the body condition of HF cows compared with GS cows. In general, GS cows are heavier cows, because they are a dual-purpose breed for

milk and meat. With starting of lactation, the required nutrients are increasing. Over conditioned cows take longer time to increase their dry matter intake and mobilize more body fat tissue instead. Subsequently, the NEFA and BHB values in blood increase in the early lactation. To prevent negative energy balance and extreme mobilization of body fat, the cows may be scored and monitored with the chart for body condition score (BCS) according to EDMONSON et al. (1989). A BCS under 3.5 is recommended for calving (GILLUND et al., 2001; BUSATO et al., 2002).

In our study, the risk ratios for elevated BHB for HF cows with 1.1 are lower than for GS cows with 1.46. More significant is the difference of risk ratios for elevated NEFA with lower risk ratio of 1.51 for HF and of 3.38 for GS cows. These findings underscore again the above-mentioned reasons for the lower prevalence in HF than in GS cows. Conversely, high producing cows are more likely to have metabolic imbalances. According to WALLESER (2020), the high producing cow often shows elevated BHB values and varying NEFA values. The question is, if they are able to balance the elevated metabolites and to adapt their metabolism to the requirements of lactation.

There is an increased prevalence in older high producing cows with an incidence of 43 % compared with primiparous cows with 16 %, mentioned by Mc ART et al. (2012). This seems to be obvious, because milk production of heifers is usually lower than in multiparous cows and they are often over conditioned at their first parturition. The risk ratios of elevated BHB and NEFA values separately analyzed for heifers and cows in our study do not confirm these findings. They present no significant difference in their risk ratios. The risk ratios vary depending on the DIM, but not depending on the number of lactations. However, the variable “heifer_cow” in the predictors ranking, appears with range 47.24 and 10.26 on the fourth position in the BHB models, and with range 57.88 on the third position in the model NEFA#2, as presented in Table 1. In the ranking of NEFA#1, the variable “heifer_cow” was not considered. The prediction models of our study could be used with equal performance quality for primiparous and multiparous cows, respectively. In summary, the impact of the individual metabolic situation is higher correlated than the phenotypical characteristic “heifer_cow”.

3.2. Impact on milk production

One reason for the vulnerability of high performing cows are the increased nutrient requirements compared with cows with lower milk yield. These cows are an economic risk for their farms, because the milk production is decreasing before the diagnosis of ketosis is usually made. The average loss in milk has been numbered in literature with 112 kg (Standard deviation 89 kg) over the whole lactation period of 305 days (RABOISSON et al., 2014). As the peak in milk reduction is to expected two weeks after parturition with 3 - 5.3 kg per day (RAJALA-SCHULTZ et al., 1999), it is worth to predict affected cows as early in the lactation as possible. The results showed prevalence risk ratios for elevated BHB and NEFA values and subsequently an optimal sampling time between DIM 7 - 13 for Holstein Friesian and between DIM 7 - 20 for German Simmental cows. The characteristic of the cow type “athletic cow” underscores this theory. This group has high milk yields and high BHB values, but is able to recover in a short time. Their milk yield decreases within the phase of elevation of BHB, but is still similar to the production of healthy cows with originally lower milk yields. The “clever cows” do not reduce their milk yield in quantity, but reduce the milk fat to safe energy.

The imbalances in metabolism occur in the early lactation and could be detected with the presented tool. The prediction of elevated values of BHB and NEFA in milk can help to adapt the management decisions and to start modifications in feeding to avoid further consequences. Additionally, ITB requires reliable values to have evidence for their recommendations and severe monitoring and treatment of cows at risk. For example, one dairy farm in our study recognized elevations in the BHB and NEFA values in our weekly samplings over few months. They analyzed their feed and detected a lot of hay with reduced energy value. They replaced the hay and the values in BHB and NEFA returned to normal. This is an example for practical herd health monitoring with concrete results and improvements.

3.3. Financial impact

A strong argument to predict cows at risk at an early stage, is the financial impact if the affected cows remain undetected. These cows do not only reduce their milk yield but also are at higher risks for several production diseases (e. g. displaced abomasum, retained placenta, metritis) and impaired longevity. Authors used to calculate in several financial models the costs for each case of hyperketonemia. They presented cost per case between $ 203 and $ 289 in different studies (RABOISSON et al., 2014; MC ART et al., 2015; GOHARY et al., 2016). These costs need to be compared with expected costs of routine milk sampling for herd monitoring and predicting cows at risk. It is an economic decision for the dairy farmers, but also a decision for animal welfare and protection of health. Further studies are required to evaluate and estimate the actual cost reduction by less cases of metabolic diseases compared to additional costs through the regular milk tests and application of the prediction models. A study that applies the prediction in different cow groups with the recommended weekly testing, longer intervals between the testing, and no testing as control group could help to number the reduction of costs through early detected cases and the occurring costs by regular testing. A numeric outcome of the prediction model could also help to draw a herd profile and target a demand-oriented testing that could be used as additional service of the LKVs.

3.4. Diagnostic methods

The first publication presents several diagnostic approaches to detect cows with disturbed metabolism. The direct cow-side ketosis tests are suitable for suspicious animals but not for overall testing in herds. The enlargement of automatized working processes makes it difficult to identify suspicious cows early, because the contact between farmer and cow is reduced. Therefore, a screening tool with features for herd monitoring is helpful to predict and identify cows at risk for metabolic imbalances. Suspicious cows could be tested with a cow-side test such as test stripes for urine with a Se of 78 % [Standard Error (SE) 6.0] and a Sp of 96 % (SE 0.8) (Ketostix®, Bayer Corporation, Elkhart, IN, USA). For milk, there are test stripes (KetoTest™, Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, JP) with Se of 73 % (SE 5.5) and a Sp of 96 % (SE 0.7) (CARRIER et al. 2004). Hand-held instruments as the Precision Xtra® (Abbott Diabetes Care, Abingdon, UK) for blood test show a Sp of 100 % (CI 94 - 100 %) and a Se up to 100 % (CI 69 - 100

%) (IWERSEN et al., 2009). For the recommended herd screening, FTIR spectroscopy is a suitable technology to detect cows with elevated BHB and NEFA values in routine milk samples (VAN DER DRIFT et al., 2012; VAN KNEGSEL et al., 2010). The study of VAN DER DRIFT et al. (2012) presents similar preconditions and findings compared with our study. They combined FTIR spectra with cow information and received a Se of 82.4 % (CI 77.0 - 87.9 %) and a Sp of 83.8 % (CI 81.9 - 85.6 %), respectively. In a long-term study of SANTSCHI et al. (2016), the implementation of routine milk testing with FTIR spectroscopy in the Dairy Herd Improvement (DHI) program was tested. They defined thresholds to identify suspicious cows and received a Se of 96 % and a Sp of 89 %. Their detected prevalence is with 22.6 % similar to the prevalence of 23 % in our study.

Based on the results of several studies, especially the studies of TREMBLAY et al. (2018, 2019), the development of a prediction model based on FTIR data that could be used in a routine setting started. The aim was to provide a tool that is able to compensate the low prevalence of the elevated values and the imbalanced data sets. To receive a prediction model that is compatible for a broad audience, several model options needed to be considered and compared. Therefore, TREMBLAY et al. (2018) applied FTIR data from German Simmentals, collected in the FSM-IRMI project of the Clinic for Ruminants (LMU), on rtFMS approach. They decided to use this method to compare different model options in a regression tree and find out the best performance.

4. Early warning system for HYK and PMAS

4.1. Prediction models based on regression tree full model selection

The rtFMS approach provides transparency of the modeling and the different options. The user is able to choose between different modeling options. The included input variables were prioritized and the one with the least variance in the value of balanced accuracy was included in the tree in descending order. The branching was terminated when no statistically significant difference between input variable and outcome existed. The terminal node contained balanced accuracy as measure of performance of the combined modeling options. Balanced accuracy is a parameter that is suitable for imbalanced data and more flexible than others (JAPKOWICZ AND STEPHEN, 2002). The data need to be balanced

because rare events, less than 15 % of observations, render accurate predictions difficult. But the synthetic minority oversampling technique (SMOTE) presents itself as a suitable method to balance the data and to make them more proper for modeling. SMOTE is described in detail by CHAWLA et al. (2002). The decision for the optimal model is dependent on the resulted balanced accuracy, personal preference, and the statistically indistinguishable 95 % CI. The evaluated approach is suitable for the application on FTIR data to derive specific models for the individual requirements of the user by selection of individualized input variables. The utilization and implementation in existing routine analysing processes as milk performance test is possible due to the stepwise modeling development (TREMBLAY et al., 2019).

Based on this rtFMS method, the two recommended models for BHB differ in the input of fatty acid panels. The performance is more accurate if those panels are included, but there is also an alternative model. The NEFA prediction models are differentiating in the implementation of the principal component analysis (PCA) that is used to group associated variables (SMITH LINDSAY, 2002).

The outcome in our study is binary, above the determined thresholds or below, but a prediction model with numeric output is developed for predicting exact values of BHB and NEFA in the FTIR data. A numeric prediction improves the value of prediction and the identification of the individual cow group. Veterinarians are able to differentiate numeric prediction values in several categories of risk and affection, respectively, and not only in positive or negative predictions.

4.2. Ranking the impact of variables on the prediction models

To range the impact of variables on the prediction quality, the Pearson`s correlation coefficient was used. If the coefficient was more than 0.7, the variable was included in a ranking list scaled from 0 to 100. The strongest associations between prediction and variable were scaled to 100, that are various fatty acids in the NEFA models and the wavelength of fat and acetone in the BHB models. Other variables were checked on their impact on the prediction. The effect of the morning or evening time of milking, for example, was not supported by the low position of the variable “AM_PM” in the ranking list. SCHWENDEL et al. (2015) showed higher amounts of C4:0 to C16:0, saturated fatty acids, in milk samples taken in the morning (AM samples). In contrary, milk samples of the evening (PM

samples) have a greater share odd- and branched-chain fatty acids, vaccenic acids, oleic acids, and conjugated linoleic acids. Additionally, protein, fat, urea, and lactoferrin are increased in AM samples compared with PM samples (EISENBERG et al., 2016). The variable *"*heifer_cow*"* presented itself on position three and four in the ranking. This makes an impact of the parity on the prediction quality likely. In contrast to that are the prevalence risk ratios, calculated for heifers and cows separately, which do not confirm the results of the predictors ranking. The prevalence risk ratios for elevated BHB or NEFA values were similar and do not make a differentiation between heifers and cows in the prediction models necessary. The predictors ranking is a tool to provide transparency and weigh the variables that need to be investigated more detailed. The ranking allows for the evaluation of input variables for different prediction models. The prediction models developed with rtFMS are different in their modeling options, depending on the data set and the selected outcome values. With the predictors ranking the different input variables can be compared and the evaluation of their impact on the models' performance is visualized. This provides transparency and comparability for application of the models with different input options and requirements.

4.3. Impact of variable "breed" on the prediction

An important question in the validation of the prediction models was if the differentiation between breeds for applying the prediction model is required. Therefore, the main dairy breeds in Germany, Holstein Friesian (2000 cows) and German Simmental (541 cows) were investigated in our study. The constitution of both breeds is different and their metabolism as well. To identify differences in the prediction quality or performance, the FTIR data was separated in two breed-related subsets. The models were applied simultaneously on these subsets to achieve comparable results. The modeling with the stratified data resulted in overlapping confidence intervals and confirmed that the prediction models are suitable for both kinds of breeds. The study of TREMBLAY et al. (2019) investigated cows in DIM 5 to 50 for elevated BHB and NEFA values and associations with clinical symptoms. They used FTIR data from weekly gained milk samples and corresponding blood samples. In contrast to our study, their data contained a majority of German Simmental cows and only three cows of the Holstein Friesian breed. They identified a significant association between

elevation of NEFA values and clinical symptoms and identified the poor metabolic adaptation syndrome (PMAS), but a point of criticism was the missing variety in breeds. The identification of potential differences among breeds is necessary for the practical implementation, because there are often breed mixed herds. Our findings show that both breeds may be applicated on the prediction models equally.

4.4. Evaluation of the optimal sampling time and frequency

The findings regarding the optimal sampling time are required for the realization in the field as well. Literature documented the early lactation as the time with the highest prevalence of metabolic imbalances. The results in our study revealed the peak in prevalence between DIM 7 – 13 for Holstein Friesian and between DIM 7-20 for German Simmental. The prevalence of elevated BHB and NEFA values was at its top between DIM 7 and 13 with 13 % for all cows. It decreased throughout the lactation towards 2 % between DIM 42 and 50. We assume that cows with increased values which occurred in weeks with lower prevalence also are part of the cows showing increased values in all weeks with higher prevalence. With a weekly testing, each animal is sampled at least once in DIM 7-13 and almost all cows at risk can be detected (100 % hit, 0 % loss). The missing rate is the part of affected cows which are not detected because of the time passing between the milk samples. This calculation is ongoing until a sampling every five weeks, with a hit rate of 65.1 % and a missing rate of 34.9 %. The results regarding the sampling frequency were urgently expected. The recommended frequency lead to arguments for the implementation of milk recording services by the LKVs. The American DHIA provides different services. There are for example the monthly routine testing and an additional testing for fresh cows from DIM 7 to 13 that is invoiced separately. DHIA services are covering 46 % of the dairy farms in North America, whereas the DLQ, head of the German DHIA`s, covers 88 % of the German dairy farms with their monthly milk recording test. The German Dairy Herd Improvement Associations need to evaluate if there is enough requirement for such a service in the market, because to provide the milk testing weekly or in a customer referred frequency, the sampling logistic and the transport to the laboratories and the analysis and the reporting to the dairy farms need to be organized. A customer-friendly milk testing service could also continuously support the improvement of the management and the animal health

of dairy farms (DAIRY HERD INFORMATION ASSOCIATION, 2020). The integrated veterinary herd health care could use the provided data to secure a herd health monitoring in an integrated form and to support the dairy farms with recommendations according to their detected metabolic health status.

Within the field trial of the Q-Check study, the dairy farmers appreciated the weekly testing and monitoring of the cows in the early lactation. The report was provided a few days after sampling and allowed for a prompt reaction to the occurrence of metabolic imbalances. The short time between sampling and reporting is paramount for the efficiency of a routine monitoring. The metabolic values and the energy status of the cows are changing rapidly in the early lactation. This needs to be considered for the implementation in the existing structures of milk performance tests.

4.5. Impact of standardization of infrared spectral data

Other projects, such as OptiMIR standardization, developed processes and tools to improve FTIR data before. It was a European project to equalize FTIR data from different analyzers to create comparable results. Our study was not suitable to evaluate whether standardization of data is necessary, because the study design only used one FOSS Milkoscan 7 RM to produce the FTIR data. Therefore, the result was of course that data with OptiMIR standardization versus non-standardized data showed no significant differences in their results. The European approach to develop prediction tools and preparation processes for FTIR data with a data base for standardized FTIR data presents the importance of this topic for the agriculture and economy in Europe (GRELET et al., 2017).

VI. SUMMARY

The fundamental idea of the project “Q-Check – Animal welfare in dairy farming – from self-assessment to national monitoring by system” was to realize concrete and practical improvements for dairy cows. The veterinary expertise in the project was required in working package III, the development of an early warning system for metabolic disorders. To implement the project goals, the compliance of dairy farmers and other stakeholders of the milk producing industry was required. Subsequently, the first part of the project contained a literature research to draw an overview of the reasons and arguments that underscore the requirement of herd health monitoring. There are strong veterinary and economic arguments to put effort in a routine monitoring. The financial impact through associated diseases, death, and loss in milk production need to be considered as well as the change in agricultural structure and the enlarged data sources that are available. The evaluation of different diagnostic technologies recommended the Fourier transform infrared spectroscopy (FTIR) of milk samples as a suitable technique for detecting metabolic disorders in early lactation. In summary, the publication “Herd health monitoring in dairy farms – discover metabolic diseases. An overview” presents the main result that metabolic health and animal welfare of dairy cows could be improved by routine herd health monitoring.

This was the initial situation for pursuing research and the second part of the project. A field study to collect blood and milk samples from 2,541 cows in the early lactation period was organized and carried out. The delivered data were used for the development of a prediction model to provide improved routine monitoring through FTIR spectroscopy. According to previous studies, the regression tree full model selection (rtFMS) approach was chosen to develop the prediction model. The possibility to compare different modeling options and to make the impact of different variables transparent for the user, is a strong advantage of this method. The results for the final models for ß-hydroxybutyrate (BHB) and nonesterified fatty acids (NEFA) values convinced with their statistical performance. The BHB model has a sensitivity of 82.18 % [confidence interval (CI) 78.97 - 85.08 %], a specificity of 82.66 % (CI 81.88 - 83.43 %), and a balanced accuracy of 82.42 % (CI 80.42 - 84.25 %). The NEFA model presents a sensitivity of 80.68 % (CI 72.64 - 83.46 %), a specificity of 82.52 % (CI 81.72 -

83.29 %), and a balanced accuracy of 81.60 % (CI 79.68 - 83.37 %). In order to achieve a prediction tool for the practical implementation, several preconditions to optimize the application and realization on dairy farms were evaluated. The impact of morning or evening time of sampling, the impact of sampling primi- or multiparous cows, of standardization of the FTIR data, and the impact of different breeds on the prediction models were validated. A recommendation for the best day in milk (DIM) and for the optimal frequency for sampling was provided. The results are described in the second publication “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”. The recommended models for BHB and NEFA outcome values are based on FTIR data optional with fatty acids panels and use background information of the cows. The samples may be taken in the week with maximum prevalence, between DIM 7 - 13. A weekly sampling frequency is suitable to achieve the best hit rate of cows at risk. In contrary, the missing rate of cows at risk is 34.9 % with a sampling every five weeks. The prediction models may be used both for Holstein Friesian and German Simmental cows, as there was no significant difference in their prediction and statistical performance. The daytime for the sampling as well as the differentiation between heifers and cows are not required for the presented prediction models. The requirement of standardization of FTIR data is not substantiated. This is may be attributable to the utilization of a standardized infrared spectroscope within the field trial. Due to the evaluated preconditions and the transparency of the rtFMS method, the validated prediction models may be incorporated in routine settings such as the milk performance test of the German Dairy Herd Improvement Associations (LKVs). Approximately 88 % of the dairy farms in Germany could benefit from the prediction tool, hypothesizing the frequent distribution through the implementation in the routine milk samplings of the LKVs.

The aim to develop an early warning system for metabolic disorders, that is balanced in effort and benefit for the farmer was achieved. It has defined preconditions, is accurate in the prediction quality, and could be implemented in the existing processes of the routine milk performance test. The overall thesis was to recommend a method that is suitable to detect metabolic imbalances early and to enable the integrated veterinary herd health care to support responsible herd

management through the dairy farmers.

The presented prediction models on rtFMS approach, based on FTIR data from regular milk samples, considering the evaluated preconditions, supports the improvement of integrated veterinary herd health care, herd health monitoring, and animal welfare. Finally, the objective of Q-Check, to improve animal welfare on dairy farms, is realized with the early warning system and the early detection of metabolic disorders that lead to an improvement in animal health.

VII. ZUSAMMENFASSUNG

Die grundlegende Idee des Projektes “Q-Check - Tierwohl mit System – von der betrieblichen Eigenkontrolle bis zum nationalen Monitoring“ ist die praktische Umsetzung und konkrete Verbesserung des Tierwohls in Milchviehbetrieben. Die tierärztliche Expertise in diesem Projekt wird vor allem im Arbeitspaket III, der Entwicklung eines Frühwarnsystems für Stoffwechselstörungen zu Beginn der Laktation, benötigt. Um die Projektziele in der Fläche umsetzen zu können, bedarf es der Kooperation und Überzeugung der Landwirte, sowie der verschiedenen Stakeholder der milchverarbeitenden Industrie. Folglich wurde eine Literaturrecherche durchgeführt, um das Projektziel der Gesundheitsüberwachung in Herden, mit Argumenten und Begründungen zu belegen. In der Literatur finden sich zahlreiche Argumente, die ein regelmäßiges Stoffwechselmonitoring von Milchkühen aus tiermedizinischer und wirtschaftlicher Sicht rechtfertigen. Die wirtschaftlichen Belastungen durch Stoffwechselentgleisungen mit verringerter Milchleistung sowie begleitend auftretende Erkrankungen und vorzeitige, häufigere Tierverluste müssen berücksichtigt werden. In der Literatur wird die Fourier Transform Infrarot (FTIR) Spektroskopie als geeignetes Mittel dargelegt, um erhöhte Werte an ß-Hydroxybutyrat (BHB) und nicht-veresterter freier Fettsäuren (NEFA) in der Milch festzustellen. Diese Technik kommt bereits bei der Milchleistungsprüfung (MLP) der Landeskontrollverbände (LKVs) routinemäßig zum Einsatz und ermöglicht eine Implementierung eines Herdenmonitorings in vorhandene Infrastruktur. Des Weiteren führt die strukturelle Veränderung im landwirtschaftlichen Sektor zu größeren Milchviehbetrieben und zu zunehmender Automatisierung. Dadurch ändert sich der Schwerpunkt der tiermedizinischen Arbeit zu Gunsten einer integrierten tierärztlichen Bestandsbetreuung (ITB). Die Fülle an Daten, die durch die Automatisierung zur Auswertung zur Verfügung stehen, müssen für die Betriebe und die ITB nutzbar gemacht werden. Zu diesem Zweck wurde eine Übersichtsarbeit mit dem Titel “Herd health monitoring in dairy farms – discover metabolic diseases. An overview” angefertigt und veröffentlicht. Die Kernaussage dieser Arbeit ist, dass die Stoffwechselgesundheit und das Tierwohl durch routinemäßige Herdengesundheitsüberwachung verbessert werden können.

Im Anschluss wurde ein Feldversuch zur Gewinnung von korrespondierenden

Milch- und Blutproben von insgesamt 2541 Milchkühen in der Frühlaktation durchgeführt. Diese Studie lieferte die Daten für weiterführende Untersuchungen und die Entwicklung eines Vorhersagemodells von Kühen mit erhöhtem Risiko einer Stoffwechselentgleisung. Die „regression tree full model selection“ (rtFMS) Methode wurde zur Entwicklung von Vorhersagemodellen für erhöhte BHB- und erhöhte NEFA-Werte aufgrund von sehr guten statistischen Ergebnissen ausgewählt. Ein weiterer Vorteil dieser Methode ist neben der hohen Vorhersagegenauigkeit die Möglichkeit, verschiedene Optionen der Modellierung miteinander zu vergleichen. Außerdem wird der Einfluss der Variablen auf eine positive Vorhersage in einer Rangfolge für den Anwender transparent dargestellt. Die statistischen Parameter für die Vorhersagemodelle überzeugten durch ihre hohe Qualität. Das BHB-Modell hat eine Sensitivität von 82.18 % [Konfidenzintervall (CI) 78.97 - 85.08 %], eine Spezifität von 82.66 % (CI 81.88 - 83.43 %) und eine Vorhersagegenauigkeit (balanced accuracy) von 82.42 % (CI 80.42 - 84.25 %). Das NEFA-Modell hat eine Sensitivität von 80.68 % (CI 72.64 - 83.46 %), eine Spezifität von 82.52 % (CI 81.72 - 83.29 %) und eine Vorhersagegenauigkeit von 81.60 % (CI 79.68 - 83.37 %).

Um eine praktische Umsetzung des Frühwarnsystems auf Milchviehbetrieben zu ermöglichen, wurden entsprechende Voraussetzungen und Bedingungen die Probennahme betreffend evaluiert. Der Einfluss der Tageszeit der Probennahme, Unterschiede zwischen primi- oder multiparen Kühen sowie Unterschiede zwischen Tieren der Rassen Deutsche Holstein und Deutsches Fleckvieh als auch der Einfluss von standardisierten FTIR Daten auf die Ergebnisse wurden untersucht. Der optimale Zeitraum zur Probennahme und ein geeignetes Probennahmeintervall wurden validiert. Die Ergebnisse werden auch im zweiten Manuskript “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” beschrieben. Zusammenfassend basieren die validierten und empfohlenen Modelle auf FTIR Spektraldaten, optional mit Fettsäuremustern und enthalten tierspezifische Informationen. Die Proben werden optimalerweise in der Laktationswoche mit der höchsten Prävalenz, zwischen dem 7. - 13. Tag in Milch (DIM) gewonnen. Die wöchentliche Probennahme eignet sich am besten, um möglichst viele Tiere mit erhöhtem Risiko einer Stoffwechselstörung zu identifizieren. Bei einer

Probennahme im fünfwöchigen Abstand steigt die Rate der Tiere, die nicht rechtzeitig als Risikotiere erkannt werden auf 34,9 %. Die validierten Modelle können für die Rassen Deutsche Holstein und Deutsches Fleckvieh gleichermaßen verwendet werden, da keine signifikanten Unterschiede in der Vorhersagegenauigkeit und in der Qualität der statistischen Parameter bestehen. Die Berücksichtigung der Tageszeit der Probennahme und die Differenzierung nach primi- und multiparen Kühen ist in den vorgestellten Vorhersagemodellen nicht erforderlich. Die Standardisierung der FTIR Spektraldaten war in dieser Studie für die Ergebnisse nicht relevant, da die gesamten Milchproben an einem standardisierten Infrarotspektroskop analysiert wurden. Das beschriebene Frühwarnsystem, bestehend aus Vorhersagemodellen und Probenahmebedingungen, könnte in die bestehende Infrastruktur und in die Prozesse der Milchleistungsprüfung durch die LKVs implementiert werden. Dadurch würden etwa 88 % der deutschen Milchviehbetriebe von der Einführung eines Frühwarnsystems für Stoffwechselerkrankungen profitieren.

Das Ziel, ein Frühwarnsystem zu entwickeln, das mittels evaluierter Rahmenbedingungen und Voraussetzungen, einer hohen Vorhersagegenauigkeit der rtFMS-Modelle und der Implementierung in bestehende Prozesse einen großen Nutzen für die Milchviehbetriebe darstellt, wurde erreicht. Die Validierung einer Methode, die geeignet ist, Stoffwechselentgleisungen frühzeitig zu detektieren und die Zusammenarbeit zwischen tierärztlicher Bestandsbetreuung und verantwortlichem Herdenmanagement durch die Landwirte zu fördern, wurde erfolgreich umgesetzt.

Das Frühwarnsystem, basierend auf regelmäßig erhobenen FTIR Spektraldaten aus der Milch ist geeignet, unter Berücksichtigung der untersuchten Voraussetzungen die integrierte Bestandsbetreuung, das Tierwohl und die Herdengesundheit zu verbessern.

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IX. APPENDIX

Appendix 1: SOP – Blood sampling Bavaria/Blutprobennahme in Bayern

Tiere zwischen 5. und 50. Laktationstag durch Tierarzt (TA) ausgewählt	Die Auswahl der Tiere erfolgt direkt im Herdenmanagementprogramm am PC des Betriebs. Ausdruck einer Probenliste. Anhand der Ohrmarken- oder Halsbandnummer werden die Tiere vom Tierarzt (TA) identifiziert. Tiere, von denen auf Grund Erkrankung keine Milchprobe vorliegt werden ebenfalls beprobt.
TA fixiert die Tiere	Die relevanten Tiere werden vom TA entweder im Fressfanggitter, der Liegebox oder mit einem Kopfhalfter fixiert.
⇩	
Vorbereitung zur Blutproben-entnahme durch TA	Reinigung und Desinfektion der Schwanzunterseite mit Zellstoff und 70%igem Alkohol durch den TA.
Punktion der Schwanzvene durch TA	Punktion der Schwanzvene in der Medianen zwischen zwei Wirbelkörpern auf Höhe der auslaufenden Schwanzfalten mittels einer Kanüle (PrecisionGlide™, Multiple Sample Needle, 20G x 1,5“, BD) durch den TA. Korrektur der Kanüle bis die Füllung des Probenröhrchens mit ca. 8 ml, mind. 5 ml Blut (BD-Serum-Gel-Vacutainer®, SST 2 advanced, 8,5 ml, BD) durch Unterdruck erfolgt. Anschließend komprimiert der TA die Punktionsstelle.
⇩	

Füllung der Probenröhrchen mit ca. 8 ml Blut, verschließen, schwenken und beschriften durch TA mit fortlaufender Probennummer, Zuordnung und Dokumentation in der Probenliste

Der TA schwenkt die Proben fünf Mal und beschriftet sie mit einer fortlaufenden Probennummer. In der Probenliste werden die Proben dokumentiert und den entsprechenden Tieren zugeordnet.

Anschließend muss die Probe für mindestens 30 Minuten aufrechtstehend gelagert werden.

Zentrifugieren nach mind. 30 Min. stehender Lagerung bei 2000 G für 10 Min., anschl. Kühlung bei 2 – 8°C

Am Ende des Betriebsbesuchs werden sie vom TA in der portablen Zentrifuge bei 2000G für 10 Minuten zentrifugiert. Die Proben werden bis zur Analyse im Labor vom TA in einer Kühlbox bei 2-8°C gekühlt aufbewahrt.

Die Kühlbox wird an jedem Probennahmetag mit tiefgefrorenen Kühlakkus (-18°C) bestückt.

Gekühlter Transport zum Labor der Klinik für Wiederkäuer durch TA, zweimal je Woche am Dienstag- und Donnerstagabend, Probenliste per Email an das Labor

Am Abend des Dienstag und Donnerstag jeder Woche werden die gekühlten Proben vom TA in das Labor der Klinik für Wiederkäuer in Oberschleißheim transportiert und dort zur Blutanalyse gebracht.

Die Probenliste mit der Zuordnung und Dokumentation der Proben wird parallel per Email ans Labor geschickt.

Untersuchung auf BHB und NEFA, asservieren einer Rückstellprobe im Labor

Die quantitative Bestimmung von BHB und NEFA im „cobas c311-Analyzer“ Fa. Roche Diagnostics wird jede Woche zwischen Mittwoch und Freitag durchgeführt. Je Blutprobe wird eine Rückstellprobe im Labor eindeutig beschriftet und asserviert.

Mitteilung der Ergebnisse per Email an TA	Die Ergebnisse werden dem TA anhand der vervollständigten Probenliste per Email übersandt. Die Betriebe werden durch den TA informiert. Eine Kopie der Ergebnisse wird an den LKV BY geschickt.

Appendix 2: SOP – Milk sampling Bavaria/Milchprobennahme in Bayern

Tiere zwischen 5. und 50. Laktationstag werden vom Betriebsleiter (BL) im Herdenmanagementprogramm ausgewählt

Das Shuttle wird am Vortag vom TA an den Betrieb gebracht. Die Milchprobenflaschen werden vom LOP des LKV BY vor Beginn des Versuchs gebracht.

Die Auswahl der Tiere erfolgt durch den Betriebsleiter direkt im Herdenmanagementprogramm am PC.

⇩

Shuttle wird vom BL mit Probenfläschchen bestückt und für 12 h alternierend tags und nachts oder 24h angeschlossen

Das Shuttle wird nach Herstellerangaben angeschlossen. Nach ca. 6h werden die bereits befüllten Probenröhrchen entnommen, Durchmischung mit Konservierungsmittel durch Schütteln und gekühlt (2-8°C) aufbewahrt.

Achtung vor Frost schützen.

⇩

Automatische Milchprobennahme, Entnahme der Probenröhrchen, Kühlung auf 2 - 8 °C,

Achtung: Vor Frost schützen!

Nach 12 bzw. 24h wird das Shuttle abgenommen und die restlichen befüllten Probefläschchen eingekühlt.

⇩

Shuttle wird vom BL nach 12 bzw. 24 h entfernt

Von allen relevanten Tieren wird durch das Shuttle mindestens eine Gemelksprobe genommen und in die Probenflaschen eingefüllt. Sollte die Probennahme durch gravierende Probleme nicht erfolgreich gewesen sein, ist Kontakt zu den TÄ aufzunehmen.

⇩

Milchprobenflaschen werden vom BL verschlossen und bei 2 - 8°C gekühlt aufbewahrt

Die Milchprobenflaschen (Typ 6845-xx, 50 ml, Bartec Benke) sind mit Konservierungsmittel beschickt. Sie müssen verschlossen und bei 2 - 8°C gekühlt zur Abholung am nächsten Tag bereitgestellt werden.

Zuordnung und Codierung durch LOP des LKV BY als Sonderproben, Kennzeichnung der Rundmagazine mit gesondertem Deckel zur Sicherstellung der Analyse am standardisierten IR-Spektroskop

Das LKV BY ist in den Probennahmeplan durch die LMU eingebunden und informiert die Leistungsoberprüfer (LOP) über die Milchprobennahme. Am Tag nach der Milchprobe werden alle Proben durch den LOP als Sonderproben codiert, eingescannt und verpackt. Zusätzlich werden die Proben mit einem besonderen Deckel und einem Aufkleber gekennzeichnet um die Untersuchung an immer dem gleichen standardisierten Gerät sicher zu stellen.

Transport zur Molkerei/ Sammelstelle durch LOP, Abgabe von leeren Probenfläschchen an BL

Anschließend bringt der LOP die Probenflaschen zur zuständigen Sammelstelle oder Molkerei. Bei jedem Besuch bringt der LOP dem BL leere Probenflaschen mit.

Per Kurier (Milchsammelwagen) gekühlter Transport zum MPR zur IR-Spektroskopie

Die Proben werden analog zur MLP per Kurier über Nacht zur Untersuchung zum Milchprüfring Bayern e. V. gebracht. Im Kurierfahrzeug werden die Proben gekühlt transportiert.

IR-Spektroskopie erfolgt am standardisierten MilkoScan 7 RM am MPR

Die IR-Spektroskopie der Sonderproben erfolgt ausschließlich am standardisierten IR-Spektroskop MilkoScan 7 RM der Fa. FOSS GmbH und die Bestimmung der somat. Zellzahl mittels Fossomatic 5000 der Firma FOSS GmbH im Labor des MPR Bayern e. V.

Mitteilung der Ergebnisse an BL, LMU durch LKV per Exceltabelle, kein MLP Bericht

Die Ergebnisse der Milchuntersuchung werden als Exceltabelle, nicht als ausführlicher MLP-Bericht dem BL, der LMU durch den LKV BY per Email mitgeteilt.

Appendix 3: SOP – Blood sampling in Thuringia/Blutprobennahme in Thüringen

Lieferung der Probenmaterealien

Alle Materealien zur Probenlogistik werden vom Labor der Klinik für Wiederkäuer vor Versuchsbeginn an die Betriebe versandt und entsprechend dem Haltbarkeitsdatum regelmäßig nachgeliefert

⇩

Online Anforderung der Probenabholung am Vortag mit Nennung der Kunden-Nr 14 2198144, Abholfenster 12 -17 Uhr, adressierte Versandaufkleber werden bereitgestellt

Am Vortag der Probennahme wird der Abholungsauftrag für ein Zeitfenster zwischen 12-17 Uhr des Folgetags online gebucht. Die Kundennummer ist14 2198144

Die Rechnung geht direkt an das Labor der LMU.

DHL

https://www.dhl.de/de/geschaeftskunden/express/versenden/abholung-buchen.html

Telefon 0180/63453001

⇩

Probennahme jeden Dienstag, Tiere der Frühlaktationsgruppe, zwischen 5. und 30. Laktationstag

Die Auswahl der Tiere erfolgt jeden Dienstag durch den Herdenmanager am PC des Betriebs. Ausdruck einer Probenliste.

Alle Tiere der Fütterungsgruppe werden beprobt. Tiere, die auf Grund von Sekretveränderungen keine Milchprobe haben, scheiden für die Dauer der Erkrankung aus.

⇩

TA fixiert die Tiere

Die relevanten Tiere werden vom TA fixiert.

⇩

Vorbereitung zur Blutprobenentnahme durch TA

Reinigung und Desinfektion der Schwanzunterseite mit geeignetem Material durch den TA.

⇩

Punktion der Schwanzvene durch TA

Punktion der Schwanzvene in der Medianen zwischen zwei Wirbelkörpern auf Höhe der auslaufenden Schwanzfalten mittels einer Kanüle (PrecisionGlide™, Multiple Sample Needle, 20G x 1,5“, BD) durch den TA. Korrektur der Kanüle bis die Füllung des Probenröhrchens mit ca. 8 ml, mindestens aber 5 ml Blut (BD-Serum-Gel-Vacutainer®, SST 2 advanced, 8,5 ml, BD) durch Unterdruck erfolgt. Anschließend komprimiert der TA die Punktionsstelle.

Füllung der Probenröhrchen mit ca. 8 ml Blut, verschließen und beschriften durch TA mit fortlaufender Probennummer, Zuordnung und Dokumentation in der ausgedruckten Probenliste

Der TA schwenkt die Proben fünf mal um die Gelpartikel in Lösung zu bringen und beschriftet die Röhrchen mit einer fortlaufenden Probennummer. In der Probenliste werden die Proben dokumentiert und den entsprechenden Tieren zugeordnet.

Anschließend muss die Probe für mindestens 30 Minuten aufrecht gelagert werden.

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Zentrifugieren der Proben nach mind. 30 Min. stehender Lagerung, bei 3000G für 5 min 20 sec.

Am Ende des Betriebsbesuches werden die Blutproben vom TA in der Zentrifuge für 5 Min 20 sec bei 3000 G zentrifugiert. Ein Benutzerhandbuch für evtl. Fehlermeldungen liegt bei.

Gekühlter, stehender Transport der Blutproben per Versand zum Labor der Klinik für Wiederkäuer. Die Probenliste zusätzlich per Email an das Labor. Erwartete Ankunft zwischen Dienstag und Donnerstag

Im Anschluss werden die Proben in Styroporboxen mit je 8 tiefgefrorenen Kühlakkus verpackt. Die ausgedruckte Probenliste wird beigelegt und per Email an das Labor geschickt. Für den Versand werden vorgedruckte Versandaufkleber bereitgestellt. Mit diesen wird das Paket adressiert und zur Abholung von DHL bereitgestellt.

Die Proben müssen im Labor der Klinik für Wiederkäuer zwischen Dienstag und Donnerstag 10 Uhr ankommen.

Untersuchung auf BHB und NEFA, asservieren einer Rückstellprobe im Labor

Die quantitative Bestimmung von BHB und NEFA im „cobas c311-Analyzer“ Fa. Roche Diagnostics wird jede Woche zwischen Dienstag und Donnerstag durchgeführt. Je Blutprobe wird eine Rückstellprobe im Labor eindeutig beschriftet und asserviert.

Mitteilung der Ergebnisse per Email an BL, TA, HM und TVL, Kopie an LKV BY und LMU

Die Ergebnisse werden dem BL, dem TA, dem HM, dem TVL anhand der vervollständigten Probenliste per Email übersandt. Eine Kopie der Ergebnisse wird an den LKV BY und an die TÄ der LMU geschickt.

Appendix 4: SOP – Milk sampling in Thuringia/Milchprobennahme in Thüringen

Ausreichend leere Milchproben-fläschchen werden vom MPR per DHL Versand an die Betriebe geliefert

Vor Beginn des Versuchs werden vom MPR ausreichend Probefläschchen unter Berück-sichtigung des Haltbarkeitsdatums an die Betriebe versandt. Die Milchprobenflaschen (Typ 6845-xx, 50 ml, Bartec Benke) sind mit Konservierungsmittel beschickt. Vor Frost und Austrocknung schützen.

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Online Anforderung der Probenabholung am Vortag mit Nennung der Kunden-Nr 14 2198144

Abholfenster 12 -17 Uhr, adressierte Versandaufkleber werden bereitgestellt

Am Vortag der Probennahme wird der Abholungsauftrag für ein Zeitfenster zwischen 12-17 Uhr des Folgetags online gebucht. Die Kundennummer ist14 2198144. Adressierte Versandaufkleber werden bereitgestellt.

Die Rechnung geht direkt an das Labor der LMU.

DHL

https://www.dhl.de/de/geschaeftskunden/express/versenden/abholung-buchen.html

Telefon 0180/63453001

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Jeden Dienstag bzw. Mittwoch erfolgt die Milchprobennahme. Tiere der Frühlaktationsgruppe zwischen 5. und 30. Laktationstag werden vom Herdenmanager (HM) im Herden-managementprogramm ausgewählt.

Die Auswahl der Tiere erfolgt durch den Herdenmanager direkt im Herdenmanagementprogramm am PC. Eine Probenliste wird erstellt und die Probenfläschchen fortlaufend nummeriert.

Milchprobennahme im Melkstand durch Mitarbeiter des TVL alternierend morgens oder abends

Die Milchprobennahme erfolgt durch Mitarbeiter des TVL alternierend morgens oder abends. Die entsprechenden Tiere werden anhand der Ohrmarkennummer identifiziert. Tiere, die auf Grund Sekretveränderung nicht gemolken werden können, werden nicht beprobt.

Zuordnung, Codierung und Kennzeichnung der Proben durch Mitarbeiter des TVL, Vorbereitung zum gekühlten Versand

Die Proben werden durch die Mitarbeiter des TVL gescannt, als Sonderproben mittels eines Aufklebers auf den Rundmagazinen gekennzeichnet. Für den Versand werden die Proben stehend in die Rundmagazine verpackt. In die Versandpakete werden 6 Kühlakkus (-18°C) gelegt. Die Probenmilch muss vor Gefrieren geschützt sein. Parallel wird eine Probenliste im adis-Format aus dem Herdenmanagementprogramm per Email an das LKV BY geschickt.

Für den Versand werden vorgedruckte Versandaufkleber bereitgestellt. Mit diesen wird das Paket adressiert und zur Abholung von DHL bereitgestellt. Das Leergut wird vom MPR per DHL wieder an die Betriebe geschickt.

Per gekühltem, stehendem Versand in den als Sonderproben gekennzeichneten Kunststoffstativen an den MPR zur IR –Spektroskopie. Probenliste mit Zuordnung an LKV BY per Email

IR-Spektroskopie am standardisierten MilkoScan 7 RM am MPR

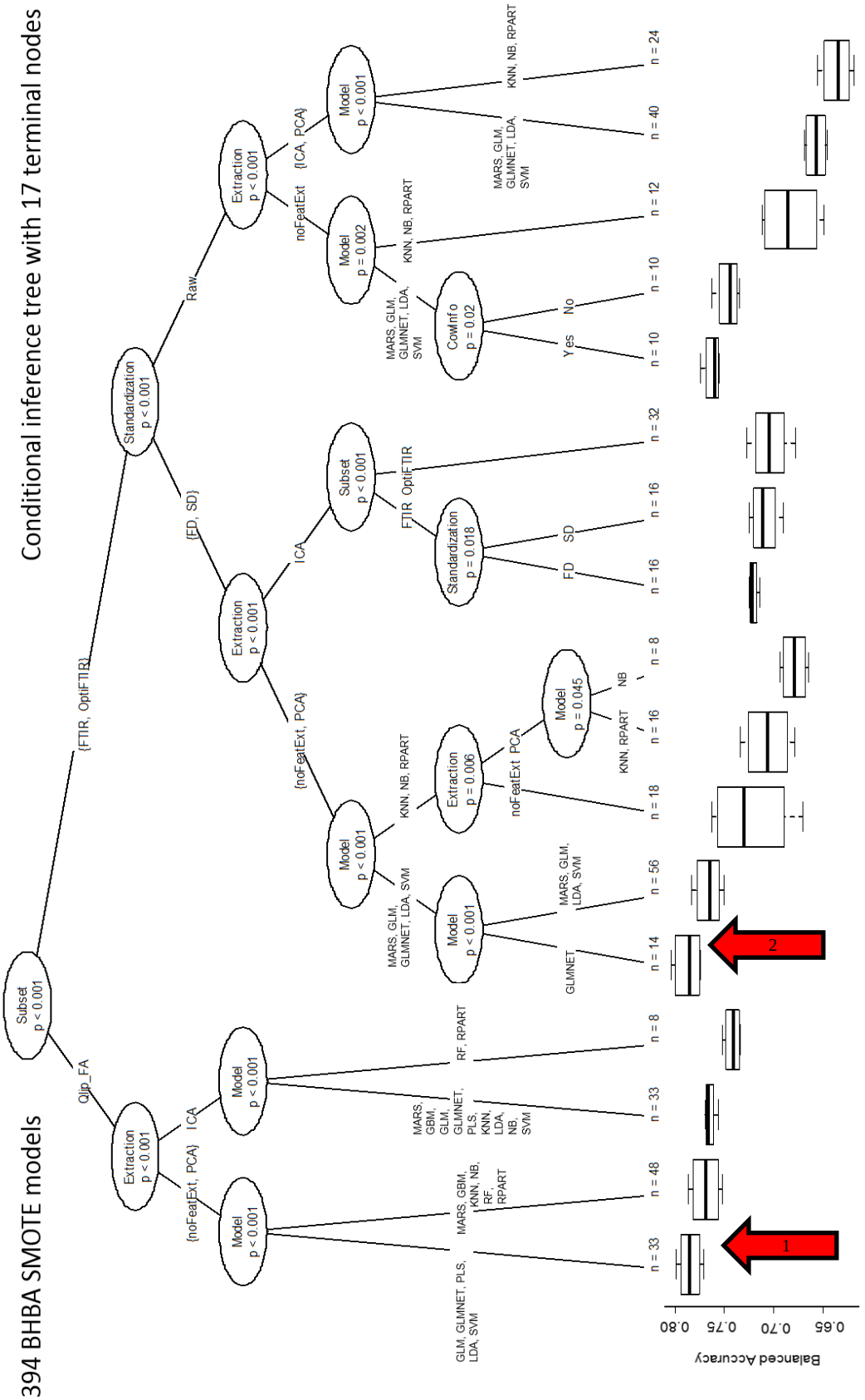
Die IR-Spektroskopie der Sonderproben erfolgt ausschließlich am standardisierten IR-Spektroskop MilkoScan 7 RM der Firma FOSS GmbH und die Bestimmung der somat. Zellzahl mittels Fossomatic 5000 der Firma FOSS GmbH im Labor des MPR Bayern e. V.

Mitteilung der Ergebnisse an BL, HM, TA, TVL und LMU per Exceltabelle, kein MLP Bericht

Die Ergebnisse der Milchuntersuchung werden als Exceltabelle, nicht als ausführlicher MLP-Bericht dem BL, HM, TA, TVL und der LMU vom LKV BY per Email mitgeteilt. Die Ergebnisse sind in das Herdenmanagementprogramm „Herde“ einlesbar.

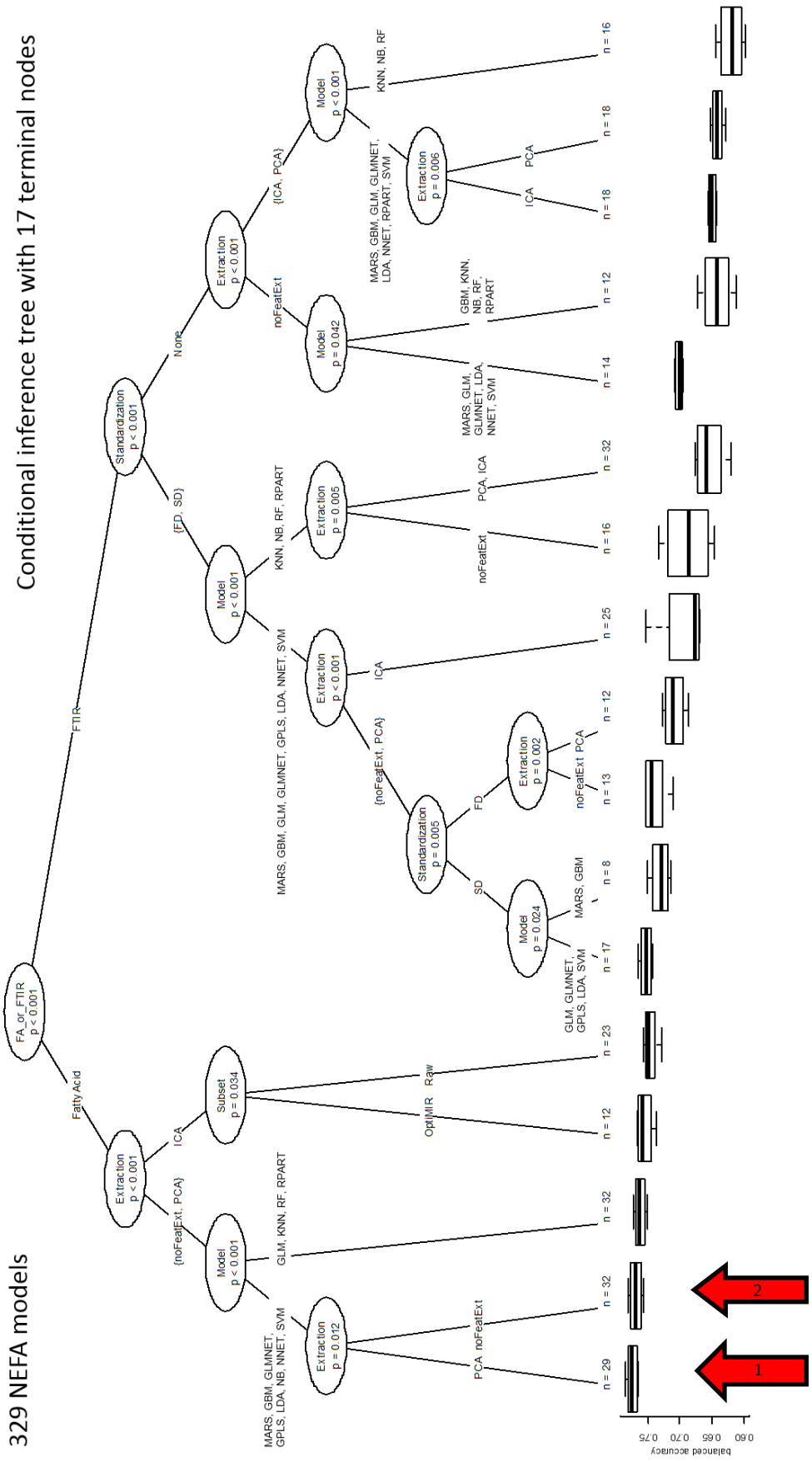
Appendix 5: Regression tree full model selection (rtFMS) results for final blood β-hydroxybutyrate (BHB) models #1 and #2

The balanced accuracies of the 394 BHB predictions models were inputted into the regression tree; n=number of models in each terminal node; boxplots visually represent the balanced accuracy of models per terminal model. The bottom and top of the box represent the 25th and 75th percentiles, respectively, and the horizontal line inside the box is the median; Subset = Milk Data Subset category; FA = fatty acid panels; FTIR = Fourier transform infrared spectroscopy; OptiFTIR = FTIR dataset with OptiMIR standardization; Stand. = Standardization category; Raw-FTIR = Raw absorbance values; FD = 1 st derivative; SD = 2nd derivative; noFeatExt. = no Feature Extraction category; PCA = Principal component analysis; ICA = Individual component analysis; Algorithm = Algorithm category; GLM = logistic generalized linear models algorithm; GLMNET = lasso and elastic-net regularized generalized linear models algorithm; LDA = linear discriminant analysis algorithm; SVM = linear support vector machines algorithm; KNN = nearest neighbour methods algorithm; NB = naive Bayes algorithm; RPART = classification trees algorithm; NNET = neural networks algorithm; GBM = gradient boosting machine algorithm; RF = random forests algorithm; MARS = multivariate adaptive regression splines algorithm; PLS = Partial least squares algorithm.

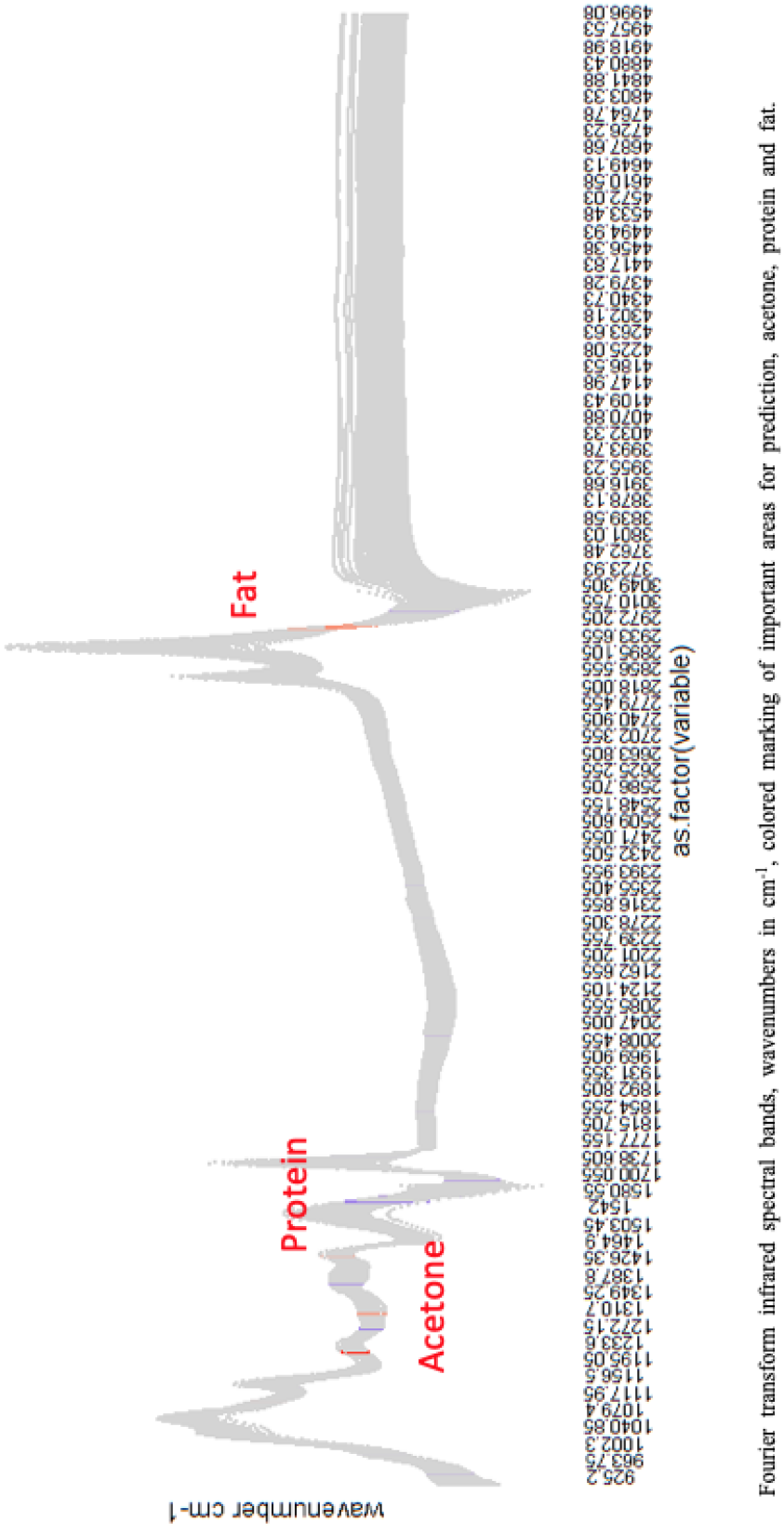

394 BHBA SMOTE models
Conditional inference tree with 17 terminal nodes
Subset
p < 0.001
Clip_FA
{FTIR, OptiFTIR}
Standardization
p < 0.001
Raw
{FD, SD}
Extraction
p < 0.001
{noFeatExt, PCA}
ICA
Model
p < 0.001
GLM, GLMNET, PLS, LDA, SVM
MARS, GBM, KNN, NB, RF, RPART
n = 33
n = 48
Model
p < 0.001
MARS, GBM, GLM, GLMNET, PLS, KNN, LDA, NB, SVM
RF, RPART
n = 33
n = 8
Extraction
p < 0.001
{noFeatExt, PCA}
ICA
Model
p < 0.001
MARS, GLM, GLMNET, LDA, SVM
KNN, NB, RPART
n = 14
n = 56
Extraction
p = 0.006
noFeatExt
PCA
Model
p = 0.045
GLMNET
MARS, GLM, LDA, SVM
KNN, RPART
NB
n = 18
n = 16
n = 8
Subset
p < 0.001
FTIR
OptiFTIR
Standardization
p = 0.018
FD
SD
n = 16
n = 16
n = 32
Extraction
p < 0.001
noFeatExt
{ICA, PCA}
Model
p = 0.002
MARS, GLM, GLMNET, LDA, SVM
KNN, NB, RPART
CowInfo
p = 0.02
Yes
No
n = 10
n = 10
n = 12
Model
p < 0.001
MARS, GLM, GLMNET, LDA, SVM
KNN, NB, RPART
n = 40
n = 24
1
2
0.65
0.70
0.75
0.80
Balanced Accuracy

Appendix 6: Regression tree full model selection (rtFMS) results for final blood nonesterified fatty acids (NEFAs) models #1 and #2

The balanced accuracies of the 329 NEFAs predictions models were inputted into the regression tree; n = number of models in each terminal node; boxplots visually represent the balanced accuracy of models per terminal model. The bottom and top of the box represent the 25th and 75th percentiles, respectively, and the horizontal line inside the box is the median; Subset = Milk Data Subset category; FA = fatty acid panels; FTIR = Fourier transform infrared spectroscopy; Stand. = Standardization category; Raw-FTIR = Raw absorbance values; FD = 1st derivative; SD = 2nd derivative; noFeatExt. = no Feature Extraction category; PCA = Principal component analysis; ICA = Individual component analysis; Algorithm = Algorithm category; GLM = logistic generalized linear models algorithm; GLMNET = lasso and elastic-net regularized generalized linear models algorithm; LDA = linear discriminant analysis algorithm; SVM = linear support vector machines algorithm; KNN = nearest neighbour methods algorithm; NB = naive Bayes algorithm; RPART = classification trees algorithm; NNET = neural networks algorithm; GBM = gradient boosting machine algorithm; RF = random forests algorithm; MARS = multivariate adaptive regression splines algorithm; GPLS = Generalized partial least squares algorithm.


329 NEFA models
Conditional inference tree with 17 terminal nodes
FA_or_FTIR p < 0.001
Fatty Acid
FTIR
Extraction p < 0.001
{noFeatExt, PCA}
ICA
Model p < 0.001
Subset p = 0.034
OptiMIR
Raw
MARS, GBM, GLMNET, GPLS, LDA, NB, NNET, SVM
GLM, KNN, RF, RPART
Extraction p = 0.012
PCA, noFeatExt
n = 29
n = 32
n = 32
n = 12
Standardization p < 0.001
None
{FD, SD}
Extraction p < 0.001
{ICA, PCA}
noFeatExt
Model p < 0.001
Extraction p = 0.001
MARS, GBM, GLM, GLMNET, GPLS, LDA, NNET, SVM
{noFeatExt, PCA}
ICA
Standardization p = 0.005
FD
SD
Extraction p = 0.002
noFeatExt PCA
Model p = 0.024
GLM, GLMNET, GPLS, LDA, SVM
MARS, GBM
n = 17
n = 8
n = 13
n = 12
n = 25
Model p = 0.042
KNN, NB, RF, RPART
Extraction p = 0.005
PCA, ICA
noFeatExt
MARS, GLM, GLMNET, LDA, NNET, SVM
GBM, KNN, NB, RF, RPART
n = 16
n = 32
n = 14
n = 12
Model p < 0.001
KNN, NB, RF
Extraction p = 0.006
ICA
PCA
MARS, GBM, GLM, GLMNET, LDA, NNET, RPART, SVM
n = 18
n = 18
n = 16
balanced accuracy
0.60
0.65
0.70
0.75
1
2

Appendix 7: Fourier transform infrared spectra with coloured marking of important bands for prediction - fat protein and acetone



Fourier transform infrared spectral bands, wavenumbers in cm^{-1}, colored marking of important areas for prediction, acetone, protein and fat.

X. ACKNOWLEDGEMENT

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