Enhancing metabolic monitoring during early lactation using NEFA in blood as additional reference indicator

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Abstract

Monitoring the metabolic situation of cows during early lactation is an important part of herd management. One parameter is the occurrence of hyperketonemia - defined as the concentration of beta-hydroxybutyric acid (BHBA) in blood above a certain threshold, e.g. ≥1.2 mmol/l. Another indicator for risk of hyperketonemia from milk analysis is the fat-protein-ratio in milk. Blood BHBA with a threshold of ≥1.2 mmol/l and a fat-protein-ratio with values ≥1.5 indicating risk of hyperketonemia were combined to one reference.

This combined reference was used to build a linear discriminant analysis (LDA) prediction model on milk mid-infrared (MIR) spectral data and information about the cow. The model predicts the risk of hyperketonemia expressed in three alert levels: Green (Low risk), yellow (medium risk) and red (high risk).

Recent research has shown that BHBA in blood is not the only important indicator for metabolic stress in early lactation. The proposed separation values for non-esterified fatty acids in blood (NEFA) of <0.39 mmol/l and ≥0.7 mmol/l were used to create a reference with three classes of low, medium and high risk of poor metabolic adaptation. An LDA model with milk MIR-spectra and cow information and this reference was built.

Farmers in Bavaria are provided with information from both models for cows in the first 50 day of the lactation with the report for each herd test day. This system helps farmers to detect potential problems and allows to intervene earlier.

New reference data for the three breeds Simmental, Brown Swiss and Holstein has recently become available from the Q Check project (q-check.org) and further data collection in Bavaria. This provided the opportunity to enhance the original models and to better evaluate their performance.

Keywords: FTIR spectra, metabolic monitoring, prediction model.

Introduction

Milk testing by a Dairy Herd Improvement Association provides the farmer with the fat-protein-ratio (FER) as a basic tool to evaluate the metabolic situation of dairy cows.

Availability of the MIR spectra for milk samples gives the opportunity to look into models which use more information as predictors for dangerous metabolic situations of dairy cows. The focus of our research was on the early lactation, i. e. 5 to 50 days in milk.

Two references were used: The concentration of for non-esterified fatty (NEFA) acids in blood and the concentration of beta-hydroxybutyric acid (BHBA) in blood combined with the FER. Blood samples were used because they are a reliable analytic standard.

High NEFA values are associated with the body fat mobilization due to negative energy balance and are often a precursor for hyperketonemia. Research also shows that high NEFA levels alone are an indicator for dangerous metabolic stress (Tremblay, 2018).

Since 2019 Bavaria uses a dual traffic light warning system with an early warning based on the NEFA reference and alerts based on the combined BHBA and FER reference.

This paper compares the initial models with the new models based on a much larger dataset.

Materials and methods

Blood and milk samples were collected weekly from 5 to 50 days in milk (DIM). The initial dataset contains data from 26 farms. 359 Simmental cows, 1038 with samples collected 2015-05-05 to 2016-02-11. Two projects collected data using the same protocol: Q Check (2018-01-02 – 2018-12-20) and Metalarm (2019-10-14 – 2021-02-18). A new dataset combined all data from 103 farms, 4058 Simmental, Holstein and Brown Swiss cows and 16923 samples in total.

Milk samples were analyzed on FOSS MilkoScan™ Analyzers (FOSS GmbH, Hamburg, Germany) at the Bavarian Association for raw milk testing (Milchprüfring Bayern e.V.).

Blood samples were analyzed at the laboratory of the Clinic for Ruminants in Oberschleissheim for BHBA and NEFA.

For the NEFA models reference classes were low (green NEFA <0.39 mmol//l), medium (yellow, 0.39 ≤ NEFA ≤ 0.7 mmol/l) and high risk (red, NEFA >0.7 mmol/l), For the BHBA/FER model reference classes were low (green, BHBA <1.2 mmol/l and FER <1.5), medium (yellow, BHBA ≥1.2 mmol/l or FER ≥1.5) and high risk (red, BHBA ≥1.2 mmol/l and FER ≥1.5).

MIR-spectra were restricted to wavenumbers 980 – 1580, 1728 – 1800 and 2810 – 2980 cm^{-1} to eliminate water signals and remove areas with no or redundant information. All spectra were offset corrected. For the BHBA/FER model the dataset was balanced for the reference classes with the ROSE algorithm (Menardi, 2014).

Linear Discriminant Analysis was selected as method and model quality was assessed using 10 fold cross validation. All samples from each farm were assigned to the same fold. The cross validation was run 10 times with random assignment of the data to the folds. For assignment of the predicted classes the a posteriori probability to belong to the green reference class with custom thresholds was used.

There is a large difference in the observed prevalence for the red NEFA reference, while the observed prevalence for the BHBA/FER classes are about the same in the initial and new dataset (Table 1). There are also distinct differences in the overserved NEFA reference prevalence for the breeds in the new dataset, while the differences in the BHBA/FER reference prevalence are much smaller (Table 2).

Table 1. Observed prevalence for the reference classes in the datasets.

Reference Class	NFEA Reference				BHB/FER Reference			
	Initial Dataset		New Dataset		Initial Dataset		New Dataset	
	Samples	%	Samples	%	Samples	%	Samples	%
Green	551	53	13038	78	782	75	12514	74
Yellow	277	27	2671	15	214	21	3708	22
Red	210	20	1214	7	42	4	701	4
All	1038		16923		1038		16923	

Table 2. Observed prevalence for the NEFA (left) and BHBA/FER reference (right) classes in the new dataset for each breed.

Ref. Class	NEFA Reference			BHBA/FER Reference		
	Simmental	Holstein	Brown Swiss	Simmental	Holstein	Brown Swiss
Green %	70	83	86	74	73	78
Yellow %	19	13	11	22	24	18
Red %	10	5	3	5	3	4
Samples	8604	6076	2243	8604	6076	2243

Results

Final model quality was evaluated using the percentage of correct predictions for the green reference class (prediction and reference green) and the percentage of the false red predictions (prediction red and reference green). We think these are the most important measures for the farmer because the farmer sees the predictions and not the references and needs to get an idea how reliable the predictions are.

The NFEA models build with the new dataset shows an improvement in the correct green status messages and a worsening in the false red status messages compared with the evaluation of the initial models. There are clear differences between breeds although breed specific thresholds were used.

For the BHBA/FER there are only minimal changes and the quality is the almost the same across breed due to the breed specific thresholds.

Percentage of red status messages per month

The following results use status message instead of predicted class/predictions. The figures show the percentage of red status messages for each month from 2019 to 2020 with the initial models on the left and the new models on the right.

Status messages and culling rate

To investigate the connection between status messages and culling the last milk testing day with status messages was used with a mean day in milk of 33. Culling within 30 days after the testing date counted towards the culling rates in Table 6. For evaluation of significance the table includes 99.9 % confidence for the culling rate.

Table 3. Probality thresholds and percentages of correct status messages for the NEFA model

Predicted Class	Samples	Reference green %	Reference yellow %	Reference red %
		Simmental initial dataset (yellow < 80 %, red < 5 %)		
Green	503	73	21	6
Yellow	401	41	32	27
Red	134	13	33	53
		Simmental new dataset (yellow < 77 %, red < 16 %)		
Green	6122	85	13	2
Yellow	1277	50	35	15
Red	1205	20	36	44
		Holstein new dataset (yellow < 77 %, red < 10 %)		
Green	5026	92	7	1
Yellow	635	51	36	13
Red	415	21	40	40
		Brown Swiss new dataset (yellow < 67 %, red < 4 %)		
Green	1924	92	7	1
Yellow	224	53	35	12
Red	95	25	46	28

Table 4. Probality thresholds and percentages of correct status messages for the BHBA/FER model.

Predicted Class	Samples	Reference green %	Reference yellow %	Reference red %
		Simmental initial dataset (yellow < 38 %, red < 5 %)		
Green	756	93	6	0
Yellow	179	42	50	8
Red	102	0	73	26
		Simmental new dataset (yellow < 29 %, red < 3 %)		
Green	6352	94	6	0
Yellow	1863	26	65	9
Red	389	0	65	35
		Holstein new dataset (yellow < 30 %, red < 2 %)		
Green	4433	95	5	0
Yellow	1431	17	76	7
Red	212	0	69	31
		Brown Swiss new dataset (yellow < 37 %, red < 7 %)		
Green	1734	94	6	0
Yellow	328	30	59	11
Red	181	0	65	35

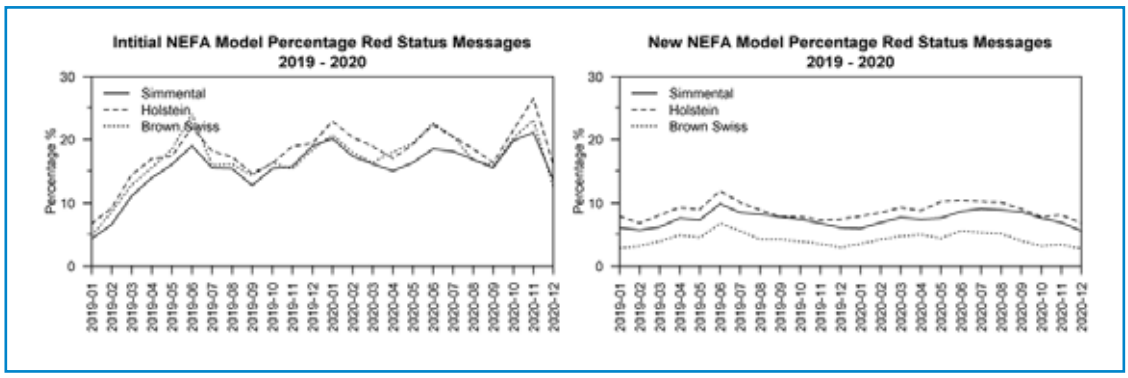

Figure 1. Percentage of red status messages of the NEFA model for each month 2019 - 2020, initial model left and new model right.

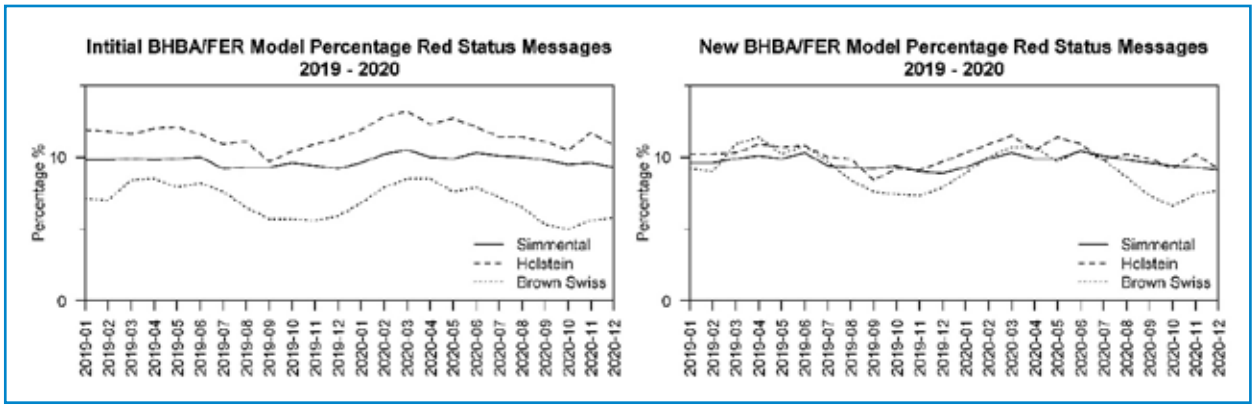

Figure 2. Percentage of red status messages of the BHBA/FER model for each month 2019 – 2020, initial model left and new model right.

Discussion

The NEFA model needed more adjustment than the BHBA/FER model. For all breed more red status messages than seem necessary were generated. Both effects can be explained by the large differences in observed prevalence. Another effect is a much less variation in the percentage over time with the model based on the new dataset. The changes in the percentage of red status messages of the BHBA/FER show the necessary adjustment for the different breed. The initial models generated more red status messages than seems necessary considering the new data, but not excessively so. As the status messages from the NEFA model were promoted as early warning we expect little impact on the farmer.

Looking at culling after the last status messages shows a significantly increased culling rate for cows with red status messages which indicates a connection between the metabolic status predicted by the models and culling.

Conclusion

New data improves our models and because of the changes in the observed prevalence also drives our model evaluation. We think that the percentage of correct predictions/ status messages is a good final evaluation measure because it gauges what the farmer will see at makes it easier to communicate. In the near future we will evaluate new model algorithms to replace linear discriminant analysis.

Table 5. Last status messages with the new models for each cow 2019-2020 and culling within 30 days.

Status message	NEFA			BHBA/FER		
	Cows	%	CI 99.9%	Cows	%	CI 99.9%
Green	989350	2.6	2.6 – 2.7	896576	2.8	2.8 – 2.9
Yellow	141826	3.4	3.2 – 3.6	213425	2.9	2.8 – 3.0
Red	84972	8.4	8.1. – 8.7	106147	6.1	5.8 – 6.3
All	1216148	3.1	3.1 – 3.2	1216148	3.1	3.1 – 3.2

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