Predicting the risk of ketosis using mid infrared spectrometry

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Abstract

Mid infrared (MIR) spectrometry is used in all milk analysis laboratories to estimate fat and protein contents. For over a decade MIR spectrometry has been used to estimate new milk components such as fatty acids, proteins and minerals. Currently, the European OptiMIR project aims to use this method to predict the physiological status of a cow, directly from its MIR spectrum. The purpose of this study was to consider the possibility of preventing ketosis by using MIR spectrometry. Ketosis is a metabolic disease caused by a disturbance of energy metabolism which affects highly productive dairy cows in high negative energy balance in early lactation. Beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) contents in blood are the gold-standard for ketosis monitoring. Indeed blood BHB and NEFA are respectively the biomarkers of energy metabolism deviation and the use of fatty reserves. A data collection took place in four experimental farms in France and Germany on 214 Holstein, Montbeliarde and Abundance cows in early lactation. Once a week from calving to 50 days in milk one blood sample and one milk sample from every cow were collected. The blood sample was analysed to measure BHB and NEFA contents and the milk sample was analysed by MIR spectrometry. Blood BHB and NEFA contents were used to classify the samples according to the risk level. 798 samples were identified as "low risk" and 421 as "high risk". The samples identified as "high risk" were then classified into three states according to the type of risk: "risk of type I ketosis", "risk of type II ketosis" and "suspected ketosis". An 81% sensitive and 69% specific prediction equation of the risk level was developed by logistic Partial Least Square (PLS) regression. In order to distinguish the type of ketosis risk a second prediction equation was developed using canonical powered PLS regression and discriminant analysis. Applied on the samples predicted as "high risk" a well classified rate of 85% could be achieved.

Keywords: mid infrared spectrometry, milk analysis, ketosis.

Introduction

The transition period after calving and the beginning of the lactation is a very critical period on which the success of the lactation is based. During this period, every cow is in negative energy balance because of the increase in the energy demand for milk production and in parallel the low intake capacity that does not allow them to cover those requirements. The more the dry matter intake decreases, the more energy the cow will

draw from the breakdown of her own body reserves. As a consequence, non-esterified fatty acids reach the liver where they are oxidised into glucose which will feed the organs (Duffield *et al.*, 1997; Goldhawk *et al.*, 2009).

When the mobilisation of body reserves becomes too intense the liver is exposed to excess non-esterified fatty acids which the cow cannot oxidise completely because of a lack of energy. This partial oxidation provides ketone bodies (acetone, acetoacetate and beta-hydroxybutyrate) that accumulate in blood, leading to a metabolic trouble called ketosis. This is particularly true in the case of high-yielding dairy cows because their demand for milk production is very high and their energy intake is often lower than required. This ailment has a lot of consequences on animal welfare and farms competitiveness because it leads to an increase in veterinarian costs and a loss of milk yield (Enjalbert *et al.*, 2001). It also modifies milk composition, especially the fat to protein ratio (Duffield *et al.*, 1997; de Roos *et al.*, 2006). We can distinguish 2 types of ketosis: the first is caused by a non-sufficient energetic density of the diet; and the second is caused by a too important body condition at calving. Two biomarkers can be taken into account to diagnose ketosis: the non-esterified fatty acids content (NEFA) in blood which relates to energy balance, and the beta-hydroxybutyrate content (BHB) in blood which is considered the gold-standard for ketosis detection (Duffield *et al.*, 1997; Oetzel, 2006). Most often the lack of symptoms leads to a poor detection of the disease. However, if you cannot diagnose it accurately, you cannot manage it well (Oetzel, 2006; Goldhawk *et al.*, 2009).

The OptiMIR project aims to develop innovative tools for milk production management through the use of mid infrared (MIR) spectral analysis of milk recording samples, which is a fast and cheap alternative method used in every lab to analyze milk composition. The purpose of this work is to determine if MIR spectroscopy could be a reliable method to monitor ketosis. The aim of the present study was two-fold. Firstly, to use both BHB and NEFA contents to classify the cows according to their risk situation for ketosis. Secondly, to predict this risk situation by using MIR spectroscopy.

Material & methods

Herds and animals

Four French and German research farms were involved in the data collection with the objective to cover a large diversity of breeds and feeding systems.

The French farm of Les Trinottières belongs to the agriculture chamber of Maine-et-Loire and is located in an oceanic lowland area of the western part of France. The cows of this farm are all Holstein cows calving in autumn and fed with a maize silage diet all year long.

The experimental farm of Marcenat belongs to INRA and is located in Auvergne Mounts, in the central part of France. In this area the climate is cold and rainy and the altitude can reach 1,000 meters. The Holstein and Montbeliarde cows calve in spring, are fed with hay and concentrate in winter and are grazing from the month of May.

The breeding center of Poisy, located in the French Savoy Mountains, belongs to the agriculture chamber of the departments of Ain, Isère, Savoie and Haute-Savoie. The Abundance, Montbeliarde and Holstein cows are calving from August to December and are fed with hay, maize and concentrates in winter and fresh grass in summer.

The Hofgüt Neumühle is located in Rhineland-Palatinate, Germany. The Holstein cows calve mainly in summer and receive a maize silage based diet all year long.

Measurements and observations

The data collection was performed on the cows from 1 to 7 weeks in milk in each herd, in the same way. In total the results of six test-days per cow (one test-day per cow per week) were stored in a common database. Samples and measurements were collected each test-day. One blood sample was collected on each cow. After centrifugation, plasma was frozen in heparinised tubes. Tubes were sent to the LDHVet laboratory where BHB and NEFA contents were determined by enzymatic method. One milk sample was collected on each cow the same day as the blood sample, for the measure of fat and protein contents and extraction of the spectrum. Each cow was weighted and body condition scored on a 5 notes scale (from 1 to 5) and their feed intake was measured. Sanitary events and overall data concerning the cows were also collected. In total 1,219 records combine a spectrum and a blood analysis (Table 1).

Table 1. Overall information on data collected per farm.

	Observations			Breed			Parity	
	Cows	Cow* test-day	Test period	HO[1]	MO[2]	AB[3]	Range	Percentage of primiparous
Marcenat	39	232	Mar-Aug 2013	49%	51%		1-8	44
Neumühle	60	358	Jul 13-Jan 14	100%			1-9	32
Poisy	49	274	Aug 13-Jan 14	6%	49%	44%	1-8	20
Trinottières	61	355	Sept 13 Jan 14	100%			1-8	43

[1] Holstein
[2] Montbeliarde
[3] Abundance

MIR spectra

In order to decrease the root mean square error and the bias due to apparatus deviation, the spectral data were standardized as described by Grelet *et al.* 2015.

Definition of ketosis status

Phenotypic correlations between the biomarkers were calculated using SAS® software. Both BHB and NEFA content values were combined into a classification in order to define the ketosis status of each observation. Thresholds used to discriminate the classes were defined on from the literature. These classes are our reference data. First, the samples were split-up into 2 classes defining the risk level: "low risk of ketosis" (LRK) versus "high risk of ketosis" (HRK). Secondly, HRK observations were split-up into 3 groups according to the type of risk: "risk of type I ketosis" (RK1), "risk of type II ketosis" (RK2) and "suspected ketosis" (SK).

Statistical models

Mathematic models were developed to assess the link between MIR spectra and ketosis status. The detection limit of the enzymatic method to measure BHB and NEFA content are respectively [0.10; 3.20] and [0.10; 3.00]. As a consequence BHB contents equal to 0.10 or 3.20 and NEFA contents equal to 0.10 or 3.00 were removed from the calibration dataset. Finally, 958 data constitute the calibration set.

The prediction equation of the risk level (LRK versus HRK) was processed by logistic partial least square regression using R software. The two thirds of the data were used for calibration and the last third was used to validate the model. The number of observations in each group was balanced in calibration dataset to avoid a distortion of the model. Sensitivity, specificity, positive predictive value and negative predictive value were calculated to assess the precision of this first model.

The prediction model of the type of risk (RK1, RK2, and SK) was developed by canonical powered partial least square regression and discriminant analysis using R software. Once again the two thirds of the dataset were used to calibrate and the last third to validate the model. Accuracy of the model was calculated to assess its precision.

Results and discussion

Phenotypic correlation between biomarkers and classification of the samples

The correlation between BHB and NEFA contents in blood is very low (0.03) as Figure 1 shows. This demonstrates the physiological difference between type I and type II ketosis risks. That is why both biomarkers were combined in order to classify the samples.

Table 2 shows the distribution of the observations across the different classes. About two thirds of the population is in the LRK group, which is consistent with the prevalence quoted in the literature (Hubbard *et al* 2010). Within the HRK group, about a half of the observations are classified as risk of type I ketosis, about 40% are classified as risk of type II ketosis and about 7% are in a situation of suspected ketosis. A third of the RK1 situations occur in the first three weeks in milk whereas only a half of the RK2 situations and two thirds of the SK situations occur in this same three-week period.

Milk yield is higher in HRK situations than in LRK situations which is consistent with the fact that ketosis is more prevalent in high-yielding dairy cows. Fat content is higher in HRK situations, especially in SK cases (49.2 g/kg in average versus 37.9 g/kg in LRK group). On the contrary, protein content is lower in SK cases, which is consistent with the lack of energy in this group. As a consequence, the average fat to protein content ratio is higher in HRK situations than in LRK situations and much higher in SK situations.

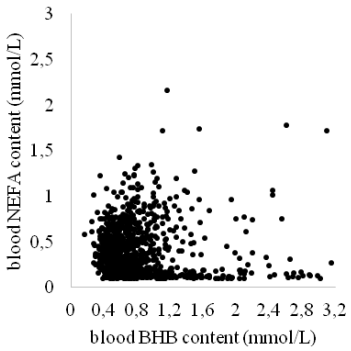

Figure 1. Phenotypic correlation between BHB and NEFA contents in blood (n=958).

Table 2. Distribution of the observations into the different classes.

	Ketosis risk level		Type of ketosis risk		
Class	LRK[1]	HRK[1]	RK1[2]	RK2[2]	SK[2]
Frequency	798	421	216	176	29
Percentage	66	35	51	42	7
MY	29.6 ± 8.3	32.9 ± 8.4	32.6 ± 7.0	33.5 ± 9.4	31.2 ± 11.3
FC	37.9 ± 6.5	41.6 ± 7.0	40.9 ± 6.9	41.1 ± 6.2	49.2 ± 8.3
PC	30.4 ± 2.9	30.2 ± 2.8	30.4 ± 2.8	30.2 ± 3.0	29.4 ± 2.7
FC / PC	1.25 ± 0.20	1.38 ± 0.24	1.35 ± 0.23	1.37 ± 0.22	1.68 ± 0.30

[1] LRK: Low Risk of Ketosis; HRK: High Risk of Ketosis
[2] RK1: Risk of type I Ketosis; RK2: Risk of type II Ketosis; SK: Suspected Ketosis
[3] MY: Milk Yield; FC: Fat Content; PC: Protein Content

367 samples were used to validate the model. Table 3 shows the results obtained on this validation dataset. The sensitivity, which is the probability of predicting HRK observations correctly, reaches 81% (78 out of 96 observations). The specificity, which is the probability of predicting LRK observations correctly, reaches 69% (188 out of 271 observations). The negative predictive value (NPV), which is the probability to be effectively observed as LRK when predicted LKR, reaches 91% (188 out of 206 predictions). However, the positive predictive value (PPV), which is the probability to be effectively observed as HRK when predicted HRK, is quite low: it reaches 48% (78 out of 161 predictions).

This model is efficient to detect HRK situations but its low PPV leads to an important proportion of false-positives. A first potential solution to explore this will be to better balance the calibration dataset.

Table 3. Accuracy of the ketosis risk level model – results on validation dataset.

	Prediction		
Observation	LRK[1]	HRK[2]	Total
LRK[1]	188	83	271
HRK[2]	18	78	96
Total	206	161	367

[1] Low Risk of Ketosis
[2] High Risk of Ketosis

Table 4. Accuracy of the type of ketosis risk model – results on validation dataset.

	Prediction			
Observation	RK1[1]	RK2[2]	SK[3]	Total
RK1[1]	65	4	2	71
RK2[2]	4	15	2	21
SK[3]	1	1	2	4
Total	70	20	6	96

[1] High Risk of type I Ketosis
[2] High Risk of type II Ketosis
[3] Suspected Ketosis

96 samples were used to validate the second model which was applied only on HRK observations. The overall accuracy of this model reaches 85%. With more details, 65 out of 71 RK1 observations (92%), 15 out of 21 RK2 observations (71%) and 2 out of 4 SK observations (50%) are well-classified, as Table 4 shows.

The model has a very good ability to detect RK1 situations. RK2 situations are also quite well detected but there are only 21 RK2 observations in the validation dataset. There are only 4 SK observations in the validation dataset so we have to be careful when interpreting the results. To improve this model, an increase in the number of records is necessary. Secondly, the calibration dataset should be balanced.

Conclusions

This study shows that predicting ketosis risk level and type using MIR spectroscopy is possible and reliable. The high sensitivity of the ketosis risk level prediction model allows us to consider the possibility to give information on animals to the farmers, to monitor and / or to treat them. What's more, the good accuracy of the type of ketosis risk

prediction model could highlight the breeding practices to check and change (e.g. energy density of the diet in early lactation, dry period management etc.). However these models have to be tested on farm to validate their accuracy on more data. To finish, the models give some alerts but do not replace clinical observation of the animals and veterinarian diagnosis.

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