Validation of a predictive model for diagnosis of high concentration of plasma non-esterified fatty acids and subclinical ketosis in dairy cows

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Introduction

Subclinical ketosis (SCK) is considered as a critical physiological disorder in dairy cows, as it is associated with reduced reproductive performance[1]. In addition, a severe negative energy balance (NEB), indicated by elevated plasma non-esterified fatty acids (NEFA), increases the risk for displaced abomasum, clinical ketosis, metritis, retained placenta, and it seems detrimental for dairy cow fertility[2]. Analysis of key blood metabolites, i.e. β-hydroxybutyrate (BHBA) and NEFA is considered the gold standard to diagnose SCK and severe NEB, respectively. However, monitoring of blood metabolites is invasive towards the cow. Hence, non-invasive milk tests are of high interest. In a previous study, we identified the milk fat (MF) C18:1 *cis*-9/C15:0 ratio and MF C18:1 *cis*-9 concentration as potential biomarkers of SCK and high blood plasma NEFA, respectively[3]. The aim of the current study was to validate the quality of the formerly developed diagnostic models using an independent dataset.

Material and Methods

In the former study, thresholds were established for the milk biomarkers to diagnose SCK or elevated blood NEFA. For this, a dataset with blood and associated milk samples were classified based on either blood plasma BHBA ($\geq$ or $<$ 0.8 mmol/L) to diagnose at risk or not for SCK or NEFA ($\geq$ or $<$ 0.6 mmol/L) to diagnose severe or mild NEB. A MF C18:1 *cis*-9/C15:0 ratio of 28 and a MF C18:1 *cis*-9 concentration of 23 allowed for a maximum false positive (FP) rate of 10%, for diagnosis of SCK and elevated blood plasma NEFA, respectively.

The current dataset was obtained from 93 cows (n = 372) in early lactation from Wageningen University Research Centre, the Netherlands. Cows were fed either a glucogenic or lipogenic diet and subjected to one of the three dry period management strategies (0, 30 or 60 days). Milk samples were collected in weeks 2, 3, 4 and 8 of lactation and analyzed for milk fatty acids, which were expressed as grams per 100 grams of fatty acid methyl esters. Blood samples were collected in weeks 2 to 8 of lactation for BHBA and NEFA analysis. To validate the MF thresholds, the dataset was split twice into two groups based on plasma BHBA and NEFA reference values (1.2 mmol/L and 0.6 mmol/L, respectively), for diagnosis of SCK and elevated blood plasma NEFA, respectively. Validation of the thresholds identified in the former study is illustrated in Table 1.

Table 1. Identification of true positive (TP), true negative (TN), false positive (FP), and false negative (FN) cases for diagnosis of subclinical ketosis (SCK) and elevated blood plasma NEFA

SCK	BHBA$\geq$1.2	BHBA$<$1.2	Elevated blood plasma NEFA	NEFA$\geq$0.6	NEFA$<$0.6
[*]C18:1 *cis*-9/C15:0 $\geq$ 28	TP	FP	[**]C18:1 *cis*-9 $\geq$ 23	TP	FP
C18:1 *cis*-9/C15:0 $<$ 28	FN	TN	C18:1 *cis*-9 $<$ 23	FN	TN

[*] ratio C18:1 *cis*-9/C15:0 in milk fat, [**] milk fat concentration of C18:1 *cis*-9, BHBA: plasma β-hydroxybutyrate, NEFA: plasma non-esterified fatty acids

From the criteria mentioned in Table 1, the sensitivity (Se), specificity (sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy can be calculated:
Se = TP/(TP+FN); Sp = TN/(TN+FP); PPV = TP/(TP+FP); NPV = TN/(TN+FN);
Accuracy = (TP+TN)/(TP+TN+FP+FN)

Results and Discussion

The prevalence of SCK and detrimental blood plasma NEFA in the current dataset was 20% (n = 73) and 12% (n = 45), respectively. A PPV of 40% for SCK diagnosis (Table 2) indicates that a cow with a positive test (i.e. MF C18:1 *cis*-9/C15:0 ≥ 28) has a 40% chance of having SCK. On the other hand, a NPV of 98% means that cows for which the test was negative (i.e. MF C18:1 *cis*-9/C15:0 < 28) only has a 2% chance of suffering from SCK. Similarly, the NPV of 94% for elevated blood plasma NEFA diagnosis indicates only 6% of the animals having a negative test (i.e. MF C18:1 *cis*-9 < 23) are having a high plasma NEFA concentration (> 0.6 mmol/L).

Table 2. Validation of diagnosis test based on milk fatty acids

Disorder	Se	Sp	PPV	NPV	Accuracy
[*]SCK	93	66	40	98	72
[**]Elevated blood plasma NEFA	69	68	23	94	68

Milk biomarker threshold values: [*] C18:1 *cis*-9/C15:0 = 28, [**]C18:1 *cis*-9 = 23

Diagnosis of SCK based on MF C18:1 *cis*-9/C15:0 resulted in Se and Sp of 93% and 66%, respectively, with an overall accuracy of 72%. The Se of a test indicates the proportion of animals identified to suffer from SCK based on the MF biomarker relative to the total number of SCK diagnosed cows based on the blood reference value. Meanwhile, Sp indicates the proportion of healthy animals, based on the MF biomarker, relative to the total number of healthy cows, based on the blood reference threshold.

Elevated blood plasma NEFA, diagnosed based on MF C18:1 *cis*-9, showed a Se and Sp of 69% and 68%, respectively, with an overall accuracy of 68%. The difference in Se between diagnosis of SCK and elevated blood plasma NEFA indicates that the model for elevated blood plasma NEFA performs worse than the SCK model.

In conclusion, validation of the formerly developed diagnostic models confirms the ratio C18:1 *cis*-9 to C15:0 in milk fat is a promising biomarker for detection of subclinical ketosis in dairy cows.

Reference
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