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Using milk components to estimate the risk of energy imbalance in Holstein cows by means of receiver operating characteristic (ROC) analysis

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Abstract: Negative energy balance (NEB) in dairy cows during early lactation significantly contributes to metabolic and infectious diseases, traditionally diagnosed via costly and time-consuming serum non-esterified fatty acids (NEFA) analysis. This study aimed to develop a practical and cost-effective diagnostic test for NEB based on milk components analysed routinely. Data from 692 Holstein cows (5–35 days in milk) located at five Czech dairy farms were analysed using multiple logistic regression and receiver operating characteristic (ROC) analysis. Results showed that 99 cows (14.3%) were classified as NEB+ (NEFA > 0.6 mmol/l). Cows in the NEB+ group exhibited a significantly higher milk fat content ($P < 0.001$) and milk fat-to-protein ratio ($P < 0.001$), and lower lactose concentrations ($P < 0.001$) compared to NEB– cows. Key indicators of lipomobilisation, such as C18:1, C18:0, and monounsaturated fatty acids (FA), were significantly higher ($P < 0.001$) in NEB+ cows, while saturated, short-chain, and medium-chain FA were lower ($P < 0.001$). The developed prediction models, incorporating milk fat and specific FA (e.g. C18:1, C18:0, C14:0), demonstrated high diagnostic efficacy. The area under the ROC curve (AUC) values ranged from 0.84 to 0.92 for individual farms and reached 0.83 for the combined dataset. Using the Index of Union method, optimal cut-off points yielded sensitivities between 0.72 and 0.86, and specificities between 0.72 and 0.85. For the overall model, both sensitivity and specificity were 0.76. In conclusion, the proposed diagnostic test, leveraging milk components, offers a reliable and practical tool for early NEB detection in dairy cows. This facilitates timely intervention, thereby mitigating adverse health and economic impacts. Further validation with larger and more diverse datasets is recommended.

Keywords: dairy cattle; diagnostic test; early disease detection; Fourier transform infrared spectroscopy; metabolic health; milk fatty acids

The most common health problems in dairy cow herds are fertility disorders, mastitis, feet and leg injuries, and metabolic diseases. This is often caused by a severe negative energy balance (NEB), which typically occurs at the beginning of lactation, when high-yielding dairy cows are unable to consume enough dry matter to meet the substantial energy demands associated with lactation and must

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thus compensate for this deficiency by mobilising their own body fat reserves (Roche et al. 2013). This physiological process, if too intense or prolonged, leads to excessive release of non-esterified fatty acids (NEFA) from adipose tissue into the bloodstream. However, dairy cows are unable to effectively metabolise excessively high blood NEFA concentrations, which is associated with a higher susceptibility to infectious diseases and risk of developing a range of metabolic diseases, such as ketosis and fatty liver syndrome, as well as displaced abomasum, mastitis and reproductive disorders (Jorjong et al. 2014; Van Saun 2016; Hussein et al. 2020; Heirbaut et al. 2023).

Serum NEFA concentrations are a reliable direct indicator of NEB intensity, as they directly reflect the extent of lipolysis. According to Van Saun (2016), a NEFA concentration greater than 0.6 mmol/l in dairy cows during the first weeks of lactation is considered as the threshold value for diagnosing NEB. Although this diagnosis is relatively accurate, it is costly, time-consuming, and logistically challenging, as it requires repeated blood sampling and subsequent specialised laboratory analysis. These limitations make it inconvenient for routine use and highlight the need for reliable, practical, and cost-effective diagnostic indicators.

In recent years, there has been growing interest in the use of milk components as indirect indicators of the metabolic status of dairy cows, as milk is an easily accessible material and its analysis is routinely performed as part of milk performance testing. It has been demonstrated that the composition of milk fat, particularly the fatty acid (FA) profile, reflects metabolic changes associated with lipomobilisation during NEB (Mantysaari et al. 2019). When NEB occurs, it is assumed that the concentrations of certain milk FAs originating from the adipose tissue increase, while the *de novo* synthesis of FAs in the mammary gland decreases due to the presence of FAs from mobilised stores (Jorjong et al. 2014; Pires et al. 2022). Furthermore, analysis of these minor milk components is easily performed using Fourier transform infrared spectroscopy (FTIR), which is fast, reliable (Soyeurt et al. 2011) and is already standard practice in milk performance control laboratories.

Assessing the diagnostic potential of indirect indicators of the metabolic status of dairy cows requires appropriate statistical tools. Multiple logistic regression and subsequent receiver operating characteristic (ROC) analysis represent modern approaches to modelling and evaluating the diagnostic accuracy of tests aimed at predicting binary outcomes, such as the presence or absence of NEB in this case. To ensure greater robustness of the prediction model, data from several farms should be used to account for differences in herd management.

The hypothesis assumed that there are reliable indirect indicators of NEB among minor milk components, particularly milk FAs, which can be used for the early prediction of NEB and the development of a diagnostic test applicable in practice. Therefore, the study aimed to develop a diagnostic test for the early detection of NEB in dairy cows, based on changes in selected milk components. This tool would be applicable in dairy herd management, enabling the early detection of NEB in dairy cows at the beginning of lactation, which would subsequently allow targeted and timely preventive measures to be taken

MATERIAL AND METHODS

Animals and herd management

The study was approved by the Animal Care Committee of the Ministry of Agriculture of the Czech Republic (No. MZE-29639/2021-18134). The data were collected from five dairy farms located in Czechia from August 2021 to November 2023. A total of 692 Holstein cows, both primiparous and multiparous, were included in the experiment. All cows were housed in free-stall barns and milked twice per day on four farms (Farms 2, 3, 4, and 5) and three times per day on one farm (Farm 1). All farms were equipped with tandem milking parlours (AfiMilk®; S.A.E. Afikim, Kibbutz Afikim, Israel). Milk production data were automatically recorded using AfiFarm™ herd management software (S.A.E. Afikim). During the experimental period, the cows were fed a total mixed ration (TMR) *ad libitum*, based on corn silage, alfalfa haylage, hay, concentrates, and a mineral-vitamin supplement. No FA supplements were included in the diets. The TMR was regularly pushed up to the cows from the feeding alley by an automatic feed pusher to ensure continuous access to feed. The animals also had free access to fresh water. Overall, herd management and feeding patterns were similar across the partici-

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pating farms. Table 1 provides an overview of the production characteristics of each farm as well as milk recording data of the farms involved in the study during the experimental period.

Sample collection and analysis

Blood samples were collected from a total of 692 cows on the same day as the routine milk performance recording. The cows included in the study were 5 to 35 days in milk. The samples were collected from the coccygeal vein using 1.20 × 25 mm single-use needles into 4 ml BD Vacutainer rapid serum tubes by trained staff at the same time on each sampling day (07:00–09:00 h) in all farms. After collection, the blood was allowed to clot and the sera were then stored at –18 °C until the analysis for NEFA concentrations. Details on blood sampling and analytical methods are described in Stolcova et al. (2024).

Composite milk samples (obtained by pooling milk from the four quarters of the udder) were collected during afternoon milking on the same day as blood sampling. Details on milk analyses can be found in Stolcova et al. (2024). Briefly, concentrations of milk fat (%), protein (%), lactose (%), milk urea nitrogen (MUN; mg/100 ml), citrate (%), individual FAs (C14:0, C16:0, C18:0, and C18:1; g/100 g of milk), and FA groups (g/100 g of milk) were determined using a MilkoScan™ Fourier IR analyser (Foss Electric, Hillerød, Denmark). The fat-to-protein ratio (FPR) was calculated using the milk fat and protein contents. The FA groups were classified [based on Application Note 64 for MilkoScan (2011; FOSS A/S, Hillerød, Denmark)] according to *i*) the length of their carbon chains: short-chain FAs (SCFA, C4–C10), medium-chain FAs (MCFA, C12–C16), and long-chain FAs (LCFA, C18 and longer); and *ii*) the number of double bonds: saturated FAs (SFA) without double bonds, MUFA with one double bond, and polyunsaturated FAs (PUFA) with two or more double bonds. Total unsaturated FAs (tUFA) were calculated as 100 – SFA. The FA concentrations were converted from g/100 g of milk to g/100 g of milk fat according to the following equation (Stolcova et al. 2024):

$$\text{FAs (g/100 g of milk fat)} = \text{FAs (g/100 g of milk)} \times 100/\text{milk fat (\%)} \quad (1)$$

Statistical analysis

Data editing and analyses were performed using SAS/STAT software, v9.4 of the SAS System for Windows (Copyright© 2002–2012; SAS Institute Inc., Cary, NC, USA).

A new binary variable was introduced based on serum NEFA concentrations with two possible values: 1 for cows suffering from NEB (NEB+, NEFA > 0.6 mmol/l) and 0 for those who were not in NEB (NEB–, NEFA ≤ 0.6 mmol/l).

The differences in milk yield and milk components between farms, and between NEB groups, were tested by ANOVA for imbalanced data, using PROC GLM. The linear model equation was:

$$Y_{ijklmn} = \mu + H_i + M_j + L_k + NEB_l + MY_m + DIM_n + e_{ijklmn} \quad (2)$$

where:

Y_{ijklmn} – observed milk yield or milk component;
μ – population mean;
H_i – fixed effect of farm i (5 levels);
M_j – fixed effect of the month of observation j (11 levels);
L_k – fixed effect of the lactation number k (2 levels: primiparous, or multiparous);
NEB_l – fixed effect of NEB status l (2 levels: NEB+, or NEB–);
MY_m – linear effect of milk yield m;
DIM_n – linear effect of day in milk n;
e_{ijklmn} – random residual effect.

Table 1. Production characteristics of herds participating in the experiment

Herd	1	2	3	4	5
Number of cows in the herd	1 020	147	490	242	294
Milk yield/day (kg)	38.2	34.5	31.5	33.2	33.2
Fat content (%)	3.75	3.81	4.30	3.88	3.73
Protein content (%)	3.44	3.40	3.68	3.51	3.44
Experimental period (month/year)	III/2023	VIII/2021–VI/2022	X/2022–I/2023	IX/2023–XI/2023	X/2021–VI/2022

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Multiple logistic regression was used to predict NEB status. A stepwise procedure under logistic regression (PROC LOGISTIC) was used to select the predictors of NEB for each farm and for the entire dataset. The evaluated predictors included milk yield, milk components, month, and days in milk (DIM) of observation, and the lactation number. The independent variables included milk yield, milk fat, protein, and lactose contents, FPR, MUN content, citrate content, individual FA contents (g/100 g of fat), and the C18:1 to C14:0 ratio. A significance level of 0.1 was required to enter a variable in the model and a significance level of 0.05 was required for it to stay in the model. The performance of selected models was assessed by their ROC curves, which plot the proportion of true positives versus the proportion of false positive events. The optimal cut-off points on the ROC curves were identified with ROCPLOT macro (SAS Support 2022) using the Index of Union (IU; Unal 2017) as a decisive criterion.

RESULTS AND DISCUSSION

Farm differences in monitored parameters

All monitored parameters are shown in Electronic Supplementary Material (ESM) Table S1. A total of 692 Holstein dairy cows from five different farms (Farm 1 to Farm 5) were included in the study, with blood and milk samples collected between the 5th and 35th day of lactation. Data analysis revealed significant differences ($P < 0.001$) between farms in milk yield and most basic milk components (fat, FPR, lactose, MUN, and citrate), except for protein. These differences reflect variability in herd management practices (e.g. milking frequency, diets, etc.) and overall farm production levels (Cobanovic et al. 2021). All monitored individual milk FAs and milk FA groups also differed significantly between farms ($P < 0.001$). The average C18:1 concentration, a key indicator of lipomobilisation, was highest in cows at Farm 4. Differences in milk FA profiles between cows from different farms are important because they influence the form of the prediction models and may, to some extent, explain the differences in threshold values subsequently determined. Serum NEFA concentrations corresponded to those observed in our previous study (Stolcova et al. 2024) and did not differ between cows from different farms, indicating similar levels of metabolic stress.

Differences in monitored parameters in dairy cow groups according to the occurrence of NEB

Determining serum NEFA concentrations is currently the most commonly used method for diagnosing the risk of NEB. A serum NEFA level higher than 0.6 mmol/l is considered as the threshold value for direct estimation of NEB in early lactation (Van Saun 2016). Of the total 692 dairy cows observed, 99 (14.3%) were in the NEB+ group. The proportion of dairy cows in the NEB+ group varied only slightly between farms; the lowest proportion was observed on Farm 3 (10.9%), while on the others it ranged around 15% (15.0–15.6%). These proportions were lower compared to some published studies; for example, Macrae et al. (2019) found NEB in 40% of dairy cows in the first 20 days of lactation in the United Kingdom, and in our previous experiment, we detected NEB in 42% of dairy cows in the first two weeks of lactation (Stolcova et al. 2020). However, it is important to note that the risk of NEB decreases with increasing days in lactation, and the current study included a wider range of days in lactation (up to 35 days). Nevertheless, even in the present study, the proportion of imbalanced cows represents a relatively significant part of the herd, which underscores the importance of the early detection of NEB.

The differences in the milk yield and milk components between NEB+ and NEB– cow groups are shown in Table 2. The milk yield from the afternoon milking did not differ between the NEB+ and NEB– groups. This finding differs from some studies that associate the presence of NEB with a decline in milk yield (Leduc et al. 2021). A possible explanation might be the compensatory ability of high-yielding dairy cows in early lactation. During this period, any energy deficit is primarily covered by the mobilisation of body reserves. This may not result in an immediate decrease in milk yield during the monitored period, but it may lead to a reduced yield over the entire lactation as body reserves are depleted (Mekuriaw 2023).

Differences in milk components reflected the presence, and physiological effects, of NEB.

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Table 2. Least square means (LSM) and standard errors (SE) for afternoon milk yield, milk composition, and fatty acid composition in the NEB-positive (NEB+) and NEB-negative (NEB–) groups

Variable	NEB+ (n = 99)	NEB– (n = 593)	P-value
	LSM ± SE	LSM ± SE	
Milk yield (kg/afternoon milking)	16.5 ± 4.88	17.5 ± 4.98	0.705
Fat (%)	4.77 ± 0.88	4.06 ± 0.81	<0.001
Protein (%)	3.35 ± 0.39	3.31 ± 0.32	0.021
FPR	1.43 ± 0.25	1.23 ± 0.23	<0.001
Lactose (%)	4.86 ± 0.23	5.00 ± 0.18	<0.001
MUN (mg/100 ml)	22.6 ± 6.75	22.2 ± 6.82	0.958
Citrate (%)	0.15 ± 0.04	0.13 ± 0.03	<0.001
C14:0 (g/100 g fat)	7.47 ± 1.43	9.09 ± 1.63	<0.001
C16:0 (g/100 g fat)	22.6 ± 2.97	25.7 ± 4.07	<0.001
C18:0 (g/100 g fat)	12.9 ± 1.70	11.2 ± 2.02	<0.001
C18:1 (g/100 g fat)	32.7 ± 4.69	28.5 ± 5.20	<0.001
SCFA (g/100 g fat)	10.6 ± 2.44	11.5 ± 2.09	<0.001
MCFA (g/100 g fat)	30.4 ± 5.18	35.8 ± 6.59	<0.001
LCFA (g/100 g fat)	45.0 ± 5.04	39.1 ± 5.60	<0.001
SFA (g/100 g fat)	58.2 ± 5.29	61.9 ± 5.32	<0.001
MUFA (g/100 g fat)	33.7 ± 3.87	29.7 ± 4.19	<0.001
PUFA (g/100 g fat)	3.24 ± 0.67	3.07 ± 0.71	0.010
tUFA (g/100 g fat)	36.8 ± 5.29	33.1 ± 5.32	<0.001

FPR = fat to protein ratio; LCFA = long-chain fatty acids; MCFA = medium-chain fatty acids; MUFA = monounsaturated fatty acids; MUN = milk urea nitrogen; NEB– = NEB-negative animals with serum NEFA concentrations ≤ 0.6 mmol/l; NEB = negative energy balance; NEB+ = NEB-positive animals with serum NEFA concentrations > 0.6 mmol/l; NEFA= non-esterified fatty acids; PUFA = polyunsaturated fatty acids; SCFA = short-chain fatty acids; SFA = saturated fatty acids; tUFA = total unsaturated fatty acids

Significantly greater (P < 0.001) concentrations of milk fat and FPR were found in the NEB+ group than in the NEB– group. A higher milk fat content was expected, as NEB mobilises FAs from adipose tissue which enter the mammary gland, where they are subsequently incorporated into milk fat (Tyburczy et al. 2008). In previous studies, there have been attempts to estimate NEB using the FPR, with FPR values higher than 1.5 or 1.3 being considered as threshold values (Gross et al. 2011; Glatz-Hoppe et al. 2020). However, using FPR alone to detect NEB is not reliable, as the results are often false positives. This is because a high FPR is found in milk with a high fat content and normal protein content (due to lipomobilisation during NEB), but it can also be found in milk with a normal fat content and low protein content, which then may indicate a nutritional problem in cows (Cabezas-Garcia et al. 2021).

The milk lactose content was significantly lower in the NEB+ group (P < 0.001), consistent with lactose synthesis being highly dependent on glucose availability, which is limited in NEB cows (Larsen and Moyes 2015). Similarly, Hamon et al. (2024) also found a decrease in milk lactose concentrations in cows with NEB, which was diagnosed using high levels of milk beta-hydroxybutyrate. An interesting finding is the significantly higher milk protein content (P = 0.021) in the NEB+ group and the absence of a significant difference in MUN content (P = 0.958). The MUN content in milk is influenced by dietary protein and energy intake and their subsequent synchronised release in the rumen (Zhao et al. 2025). Its stable level may indicate that, despite the presence of NEB, protein metabolism in the NEB+ group was not disrupted to such an extent that it manifested itself in a reduction in MUN and protein content in the present study.

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The milk citrate content was higher ($P < 0.001$) in the NEB+ group compared to the NEB– group. Citrate plays a key role in cellular energy metabolism as it is an intermediate in the citric acid cycle. It also plays an indirect role in milk FA metabolism by providing intermediates for *de novo* synthesis in the mammary gland. If *de novo* synthesis increases, milk citrate levels decrease (Garnsworthy et al. 2006). This is consistent with the current findings, as cows in the NEB– group had higher SCFA levels and lower milk citrate.

Differences in the composition of milk FAs correspond to the metabolic status of the animals and represent key indicators of lipomobilisation. The NEB+ group had lower ($P < 0.001$) levels of SFA, SCFA, MCFA, C14:0 and C16:0. Conversely, tUFA, MUFA, LCFA, C18:0, and C18:1 levels were higher ($P < 0.001$), while PUFA levels were higher ($P<0.01$) in the NEB+ group. These results are consistent with the physiological processes in cows with NEB, which undergo lipolysis, releasing NEFA into the bloodstream for energy. The NEFA, which are predominantly composed of LCFA such as C18:0 and C18:1 *cis*-9, are subsequently transported to the mammary gland and incorporated directly into milk fat (Jorjong et al. 2014; Pires et al. 2022). At the same time, the increased concentrations of LCFA in the mammary gland inhibit the *de novo* synthesis of SCFA and MCFA, as they reduce the activity of acetyl-CoA carboxylase, which is the primary regulatory step in FA synthesis (Jorjong et al. 2014). This explains the observed decrease in C14:0, C16:0, SCFA, and MCFA concentrations in the NEB+ group. However, the issue regarding C16:0 is more complex. Only approximately half of its content in milk is synthesised *de novo*, but this process is partially inhibited during NEB (Litherland et al. 2011). For this reason, C16:0 may not be a reliable indicator of NEB status.

NEB prediction based on multiple logistic regression and ROC analysis

The resulting prediction models are shown in Figure 1. Slightly different combinations of milk components were found to be significantly associated with the incidence of NEB. The milk fat content was a significant factor on Farms 1, 2, 3, and 5, confirming its role as an indicator of lipomobilisation. The content of C18:1 was a key predictor on Farms 1, 2, 3, and 4, which is consistent with previous studies that report it as an important indicator of lipolysis and serum NEFA concentrations (Heirbaut et al. 2023; Stolcova et al. 2024). The C18:0 content proved to be a significant predictor on Farms 2 and 4. However, in the case of Farm 4, it is interesting to note that the coefficient for the C18:0 variable was negative (–1.87), while on Farm 2 it was positive (+0.58).

This could be explained by specific differences in animal management, different feed compositions, or genetic factors unique to individual farms. On Farm 5, in addition to milk fat, milk lactose content (–2.63) was identified as a significant predictor, with lower levels indicating an energy deficit (Hamon et al. 2024), as well as C14:0 (–0.73), which is synthesised *de novo* and whose reduced amount indicates a suppression of its synthesis during NEB (as described above). Milk fat content, C18:0 content, and C18:1 content were selected as significant predictors in the model combining data from all farms.

To evaluate the performance of the model, ROC curves were constructed to plot the relationship between sensitivity and specificity at different cut-off points. The ROC curve is a standard tool for graphically visualising the outputs of diagnostic tests. The AUC then quantifies the overall diagnostic accuracy of the test. If the ROC curve lies above the diagonal, it means that the test correctly distinguishes between NEB+ and NEB– groups. The closer the curve is to the upper left corner (0, 1), the better the diagnostic accuracy (Corbacioglu and Aksel 2023). AUC values exceeding 0.80 are regarded as demonstrating very good discriminatory ability (Mandrekar 2010).

The AUC values for the herds evaluated ranged from 0.84 (Farm 3) to 0.92 (Farm 4), indicating the high diagnostic efficacy of the test based on milk components for predicting NEB (Figure 1). The model for all farms together achieved an AUC value of 0.83. The current results can be compared with Heirbaut et al. (2023) who also estimated the metabolic status of cows ("metabolically imbalanced cows" determined based on blood parameters) using milk components. In agreement with the present study, they observed the significance of milk FAs composition, with the highest AUC values (0.81) achieved when using a complex model including basic milk components, beta-hydroxybutyrate, and milk FAs determined either by FTIR

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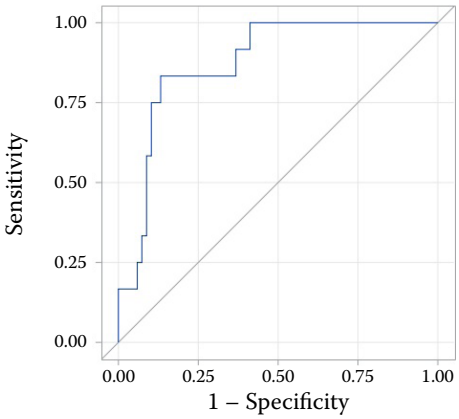
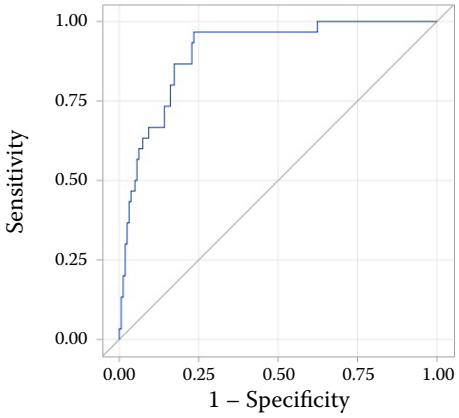
Farm 1 (N_{NEB+} = 12, N_{NEB-} = 68)



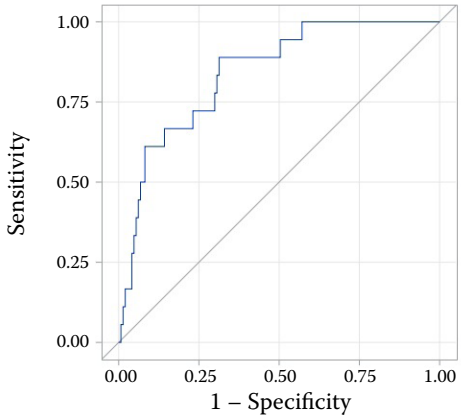
NEB = –15.73 + 1.49 × fat + 0.24 × C18:1
AUC = 0.87

Farm 2 (N_{NEB+} = 30, N_{NEB-} = 162)



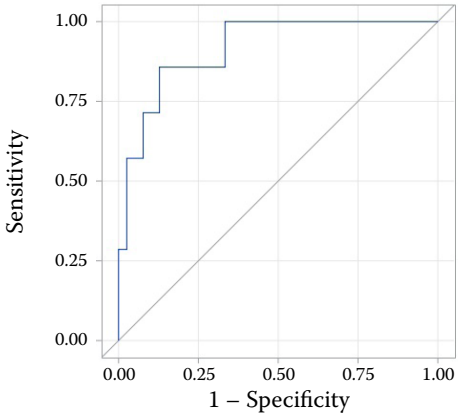
NEB = –20.52 + 0.81 × fat + 0.29 × C18:1 + 0.58 × C18:0
AUC = 0.90

Farm 3 (N_{NEB+} = 18, N_{NEB-} = 147)



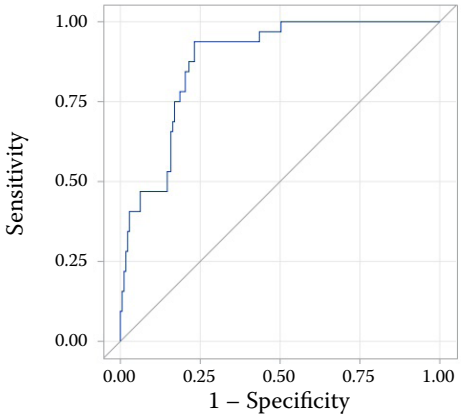
NEB = –13.32 + 0.94 × fat + 0.54 × C18:1
AUC = 0.84

Farm 4 (N_{NEB+} = 7, N_{NEB-} = 39)



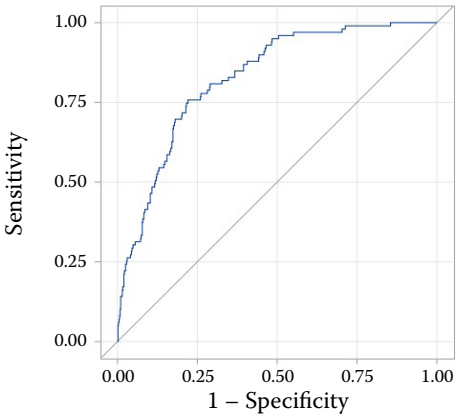
NEB = –19.62 + 1.12 × C18:1 – 1.87 × C18:0
AUC = 0.92

Farm 5 (N_{NEB+} = 32, N_{NEB-} = 177)



NEB = 10.79 + 1.21 × fat – 2.63 × lactose – 0.73 × C14:0
AUC = 0.88

All farms together (N_{NEB+} = 99, N_{NEB-} = 593)



NEB = –12.94 + 0.70 × fat + 0.30 × C18:0 + 0.14 × C18:1
AUC = 0.83

Figure 1. ROC curves, AUC, and final logistic regression models for the prediction of NEB in cows from individual farms and from all farms combined

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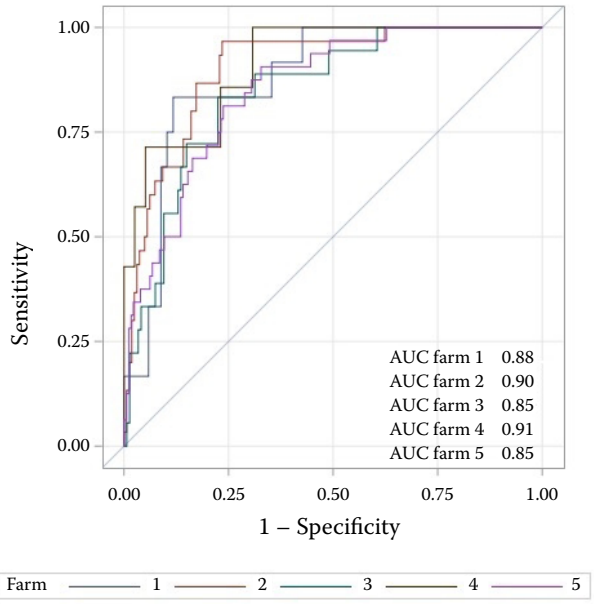

Figure 2. ROC curves obtained using the model fitted on the entire dataset from individual farms

analysis or gas chromatography. It is important to note that while Heirbaut et al. (2023) tested predefined groups of milk components as predictors, the present study utilised logistic regression to identify optimal components for each herd and the overall model.

These findings support the conclusion that milk composition is a robust and highly effective tool for monitoring and predicting metabolic health in cattle, despite the use of different analytical approaches to predictor selection.

In order to assess its general predictive ability, the model fitted to the entire dataset was applied to the data from individual farms. The resulting ROC curves and corresponding AUC are shown in Figure 2. Although the models fitted by the stepwise procedure differed slightly between farms, the generalised model performed quite well and the achieved AUC values were comparable to those from farm-specific evaluations.

Determination of cut-off values for predicting dairy cows in NEB

The advantage of ROC curves is that they can be used to determine the optimal cut-off value for quantitative biomarkers (Hassanzad and Hajian-Tilaki 2024). In accordance with recommendations in the literature (Unal 2017; Hajian-Tilaki 2018; Gerke and Zapf 2022), the IU method was used to establish the threshold values in the present study. This method utilises the absolute difference between the diagnostic rate and the AUC value to minimise the misclassification rate, exhibiting satisfactory diagnostic performance in the majority of cases (Unal 2017).

The cut-off values determined by the IU method for individual farms, and for all farms combined, together with the corresponding sensitivity, specificity, and false positive rate values, are shown in Table 3. It is important to note that in the current study, the ROC curves do not plot a single variable, but rather a set of predictors calculated based on logistic regression. Therefore, the threshold values are also the result of regression equations. The employment of a model is a more appropriate approach than the utilisation of single variable cut-off points, as it accounts for additional sources of variation (Kim et al. 2022; Heirbaut et al. 2023). Each threshold value is associated with sensitivity and specificity. For the threshold values optimised using the IU method, sensitivity ranged from 0.72 (Farm 3) to 0.86 (Farm 4) and specificity from 0.72 (Farm 3) to 0.85 (Farm 4). In other words, the utilisation of tests based on predictions derived from milk components has the potential to accurately detect 72% to 86% of cows with NEB who are truly positive, while the false positive rate (1 – Specificity) is anticipated to range between 15% and 28%. For the model that incorporates the data from all farms, the sensitivity and specificity were both 0.76. This indicates that 76% of dairy cows with NEB will be correctly identified, while 24% of healthy dairy cows will exhibit a false positive result.

Table 3. Cut-off values determined by the Index of Union method, sensitivity (Se), specificity (Sp) and false positive rate (FP = 1 – Sp) for individual farms and for all farms combined

Farm	Cut-off value (IU)	Se	Sp	FP (%)
1	0.192	0.83	0.84	16
2	0.190	0.83	0.83	17
3	0.116	0.72	0.72	28
4	0.143	0.86	0.85	15
5	0.164	0.78	0.80	20
All farms	0.152	0.76	0.76	24

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Limitations of the study

While the study demonstrates a high degree of diagnostic potential in the proposed models, it is important to acknowledge some limitations of the study. The relatively low number of cows diagnosed with NEB (n = 99) compared to the total sample, although corresponding to the actual NEB prevalence in well-managed herds, may affect the general applicability of the results. The variability of predictors between individual herds suggests that environmental factors, genetics, and feeding and housing management also play a role in the manifestation of NEB and its reflection in milk composition. Although the model created based on data from all herds is characterised by a relatively high reliability, due to the aforementioned heterogeneity of the data and the relatively small number of individuals observed, it cannot be considered as universally applicable without further validation.

CONSLUSION

The results presented in this study suggest that the proposed diagnostic test has a high potential for practical application. The ability to identify dairy cows with a NEB in a timely and reliable manner based using routinely available milk performance data would enable farmers to implement therapeutic or preventive measures (e.g. administration of propylene glycol or hepatoprotective agents) at an earlier stage.

Consequently, the implementation of early intervention strategies would enhance the efficacy of these measures, thereby mitigating the adverse effects of NEB on animal health and the economic viability of milk production. Future research should focus on expanding the database to include a larger number of animals from a wider range of farms, which would enable further validation and generalisation of predictive models. Furthermore, the integration of additional data sources (e.g. body condition or daily feed intake) into the model would enhance its prediction ability.

Conflict of interest

The authors declare no conflict of interest.

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