Predicting Metabolic Health Status Using Milk Fatty Acid Concentrations in Cows – a Review

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Abstract

Epidemiological data have established the association between increased ß-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) concentrations in blood as indicators of a metabolic disorder and of negative health, production and reproduction outcomes at both the individual cow and herd level. For both animal welfare and work efficiency reasons, monitoring dairy herds reliably for metabolic disorders through a noninvasive and automized approach is worthwhile. The aim of this review was to examine the possibility of using milk fatty acid (FA) concentrations and FA ratios to predict ketosis or metabolic disorders. Ten studies obtained from a search in two pertinent databases matched the relevant inclusion criteria. FA profiles were examined for correlations with the concentration of NEFA in blood in three studies, with the concentrations of both NEFA as well as BHB in blood in three studies and with the concentration of BHB in blood in four studies. Decreased short and medium-chain FA (C4 – C14 and C5 – C15) concentrations were associated with metabolic disorders, whereas long-chain FA (> C16) concentrations increased during the occurrence of a metabolic disorder, especially that of cis-9 C18:1. A few single-FA concentrations, such as that of cis-9 C16:1, and FA ratios, such as cis-9 C16:1 to C15:0, C17:0 to C15:0 and C18:1 to C15:0, were also correlated with a metabolic disorder. Some of these values might be useful in routine herd health monitoring despite having only moderate correlation coefficients. Two studies developed linear regression models using FA concentrations, FA ratios and other information to predict metabolic status. The implementation of refined prediction models that use all available information to predict the health status of both individual cows and the whole herd as exactly as possible might be more promising than using single FAs or FA ratios to detect cows suffering from metabolic disorders. Based on the findings of already existing and future large epidemiological studies, refined prediction models are predicted to become a supporting tool in routine herd health monitoring.

Keywords: milk fatty acids, metabolic disorder, negative energy balance, ketosis, prediction, herd health monitoring

Introdruction

Currently, the quantitative analysis of ß-hydroxybutyrate (BHB) in blood is considered the gold standard in diagnosing ketosis [1, 2]. Herdt [3] introduces the term “failure of metabolic adaptive mechanisms”, Duffield et al. [4] describe a “poor adaptive response” and Tremblay et al. [5] suggest the term “poor metabolic adaptation syndrome (PMAS)” to describe a metabolic disorder similar to ketosis. These imply that the extent of the disease is not necessarily reflected by the concentration of BHB in blood, but rather by the individual ability of the cow to adapt to the negative energy balance (NEB) that physiologically occurs at the beginning of lactation at a given point in time [6]. McArt et al. [7] and Tremblay et al. [5] describe that the concentration of non-esterified fatty acids (NEFA) in blood more reliably indicates the extent of a NEB and of the clinical symptoms of a metabolic disorder, respectively. However, epidemiological data have established the association between increased BHB and NEFA concentrations in blood as indicators of a metabolic disorder and of negative health, production and reproduction outcomes at both the individual cow and herd level [4, 8, 9].

A cheap cow-side test to quantify the concentration of blood BHB with good test performance is available [10]. Testing cows two days per week from 3 to 9 DIM (days in milk) for HYK (hyperketonemia) was the most cost-effective strategy for herds with HYK incidences between 15 % and 50 %; above 50 %, treating all fresh cows with 5 d of propylene glycol was the most cost-effective strategy in one study [11]. However, for both animal welfare and work efficiency reasons, monitoring dairy herds reliably for metabolic disorders through a noninvasive and automized approach is worthwhile. Milk is a fluid that could be potentially used for screening methods, as it is convenient and cheap to collect [9, 12]. Quick tests that measure, for example, the concentration of BHB in milk can indicate a metabolic disease but are not precise enough to reliably diagnose subclinically diseased cows [13]. Subclinical ketosis is defined as an excess level of circulating ketone bodies in the absence of the clinical signs of ketosis but with possible negative effects, such as reduced fertility [14]. Tremblay et al. [5] suggest the possibility of evaluating Fourier transform infrared spectroscopy (FTIR) data from milk for its ability to distinguish PMAS classes.

Another approach is to examine the concentrations of single fatty acid (FA) concentrations or FA ratios in milk [9, 15, 16]. An increased amount of adipose tissue is metabolized and used for milk production during states of energy deficiency [1, 17], which, in contrast to fat directly synthesized in the mammary gland, consists of long-chain fatty acids (LCFAs) [18]. Thus the milk FA profile changes during a state of NEB

[19]. This leads to the assumption that the concentration of single FAs or FA ratios could be useful in both predicting metabolic disorders and helping to understand their pathophysiology [9].
The aim of this review was to examine the possibility of using milk FA concentrations and FA ratios to predict ketosis or metabolic disorders, to evaluate the current state of research and to frame possible questions that need further research.

Material and Methods

To search for relevant publications, combinations of three terms were used in the Web of Science and PubMed databases from 1989 to 2019 to cover a wide timespan. On the one hand "milk fat composition" and "milk fatty acids", on the other hand "body condition score", "energy status", "ketosis" and "negative energy balance", moreover "hydroxybutyrate", "hyperketonemia" and "non-esterified fatty acids".
A detailed description of the review process can be found in the flow diagram in Figure 1. After the removal of duplicates, studies were selected for the screening process by reading the titles to assess their possible relevance. Screened studies were included if they were original research articles, if they used fresh dairy cows (≤ 49 DIM) and if they compared analyzed milk FAs to blood NEFA and/or BHB. Studies were excluded if the reference threshold was not in agreement with values from literature. Publications meeting these criteria were examined and interpreted.

Results

After assessing the search results for inclusion and exclusion criteria, ten studies remained (see Table 1). Except for one study using Nordic Red (NR) cows, all studies were conducted on Holstein-Friesian (HF) cows. One study used cows in parities 1 and 2, one study used cows in parity 2 and the other studies used cows in parity ≥ 2 or made no specifications. Concerning lactation stage, six studies began collecting samples in the first week, three studies in the second week, and one study in the third week after parturition.
Five studies enrolled cows fed a partial mixed ration (PMR) with additional concentrate, grass silage with additional concentrate, or a total mixed ration (TMR). The five remaining studies enrolled cows receiving various rations containing different amounts of energy.
The number of cows enrolled in the studies varied between n = 16 and n = 457, and the number of milk samples analyzed varied between n = 48 and n = 1828, with mean values of 122 cows and 572 samples, respectively. The number of samples per cow varied between n = 1.9 and n = 10, with a mean value of 5.7. Four studies used pooled samples from two consecutive milkings or over one day, four studies used morning milking samples, one study used both morning and evening milking samples and one study did not specifically describe the milking schedule.
Eight studies described using gas chromatography (GC) to determine the FA profile, two of which specified the method as gas-liquid chromatography (GLC), while two studies used Fourier transform infrared spectrometry (FTIR).
FA profiles were examined for correlations with the concentration of NEFA in blood in three studies, with the concentrations of both NEFA as well as BHB in blood in three studies and with the concentration of BHB in blood in four studies; one study additionally included metritis, displaced abomasum (DA), death and culling. For an overview of which FAs, FA groups and FA ratios were associated with an increased concentration of NEFA (NEFAhigh) or BHB (HYK), see Table 2.
Comparison of milk FA concentrations with blood NEFA concentrations: Five of the studies [16, 20-23] used plasma to determine the NEFA concentration and a threshold of ≥ 0.6 mmol/L to determine whether cows were suffering from an elevated NEFA concentration (NEFAhigh), while Mann et al. [9] used serum and a threshold of ≥ 1 mmol/L. Five studies used commercial kits to determine the concentration based on colorimetric measurement of an enzymatic reaction, and one study made no specification [22]. In four studies, blood and milk samples were collected on the same day. In the remaining studies [9, 20], blood and milk samples were collected during the same period, but blood samples were taken more frequently than milk samples. Both Dorea et al. [16] and Mann et al. [9] used univariate logistic regression for statistical analysis with area under the receiver operating characteristic curve (AUC) thresholds of ≥ 0.8 and ≥ 0.7, respectively. The accuracy of the test was calculated by generating six linear regression models (two consisting of individual FA proportions and four consisting of a ratio) that were assessed by fitting an external data set from a wider population using treatment means from literature as well as with the correct classification rate (CCR). Jorjong et al. [20] first used an exploratory discriminant analysis and a second one in which classification was based on the most discriminating milk FA. The performances were assessed through cross-validated discriminant analysis.
Mantysaari et al. [21] used individual prediction equations and the Pearson correlation coefficient. Linear regression models were developed using stepwise regression and validated through k-fold cross-validation. Puppel et al. [22] used two-way ANOVA, and Puppel et al. [23] used multivariate analysis and the Pearson correlation coefficient.
In Dorea et al. [16], ten individual milk FA proportions and four ratios reached an AUC ≥ 0.8 (see Table 3). The four ratio-based regression models separately used the ratios of C18:1 to even short- and medium-chain FAs, as well as the ratios C18:1 to C14:0, C18:1 to C15:0 and C17:0 to C15:0 and reached coefficient of determination (R^2) values of

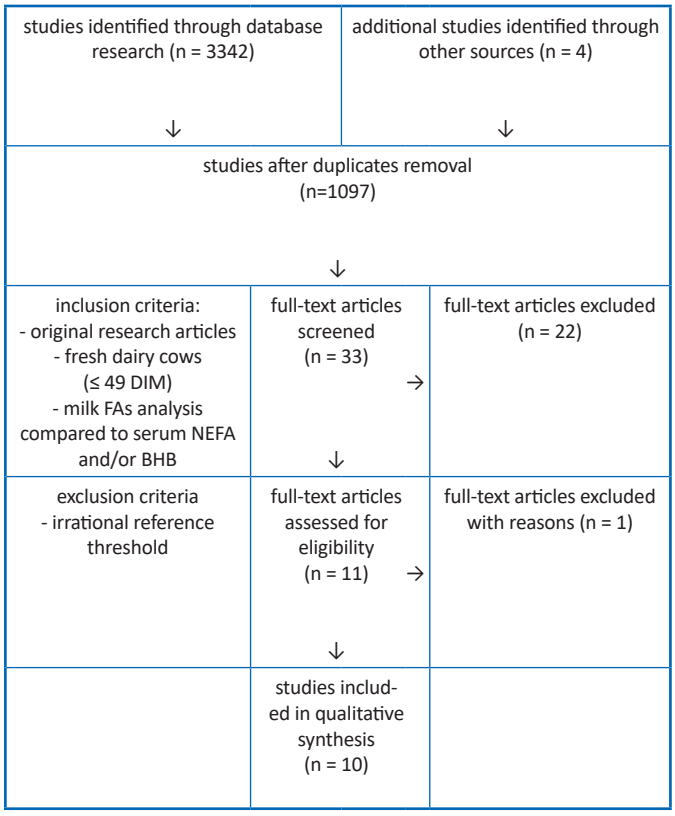

Figure 1: Flow diagram describing the review process

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Table 1: Materials and methods used in the studies considered in this review

study / parameter	Dórea et al., 2017 [16]	Jorjong et al., 2014 [20]	Mantysaari et al., 2019 [21]	Mann et al., 2016 [9]	Puppel et al., 2017 [22]	Puppel et al., 2019 [23]	Bach et al., 2019 [27]	Jorjong et al., 2015 [24]	Nogalski et al., 2015 [26]	Van Haelst et al., 2008 [25]	mean value
breed	HF	HF	NR	HF	HF	HF	HF	HF	HF	HF	-
parity	≥ 2/n.s.	≥ 2	1-2	≥ 2	≥ 2	2	≥ 2	≥ 2	n.s.	≥ 2	-
DIM	1 - 119	8 - 56	8 - 147	3 - 15	4 - 49	5 - 42	3 - 18	8 - 56	6 - 35	15 - 35	-
feed	TMR + 4 different rumen infusions/ TMR/TMR + addition of calcium salts with 2 different FA profiles	2 different diets (gluco-genic and lipogenic)	grass silage/PMR + concen-trate	TMR containing 3 different energy levels a.p. + fresh cow TMR p.p.	TMR	TMR	TMR	2 different diets (gluco-genic and lipogenic)	TMR	forage + 2 different concen-trates (glucogenic + lipogenic: glucogenic)	-
n (cows)	105	92	127	84	120	85	457	93	42	16	122
n (samples)	204	368	966	165	840	510	1828	372	420	48	572
samples/ cow	1.9	4.0	5.5	2.0	7.0	6.0	4.0	4.0	10.0	3.0	5.7
milk sample collection	pooled	morning	morning + evening	pooled	n.s.	pooled	morning	morning	morning	pooled	-
milk FA analysis	GLC	GC	FTIR	GC	GC	GC	FTIR	GC	GC	GLC	-
reference to assess metabolic disorder	NEFA ≥ 0.6 mmol/L	NEFA ≥ 0.6 mmol/L	NEFA ≥ 0.6 mmol/L	NEFA ≥ 1.0 mmol/L, BHB ≥ 1.2 mmol/L	NEFA ≥ 0.6 mmol/L, BHB ≥ 1.2 mmol/L	NEFA ≥ 0.6 mmol/L, BHB ≥ 1.2 mmol/L	BHB ≥ 1.2 mmol/L, metritis, DA, death, culling	BHB ≥ 1.2 mmol/L	BHB ≥ 1.2 mmol/L	BHB ≥ 1.2 mmol/L	-

HF = Holstein Friesian, NR = Nordic Red, DIM = days in milk, n.s. = not specified, TMR = total mixed ration, FA = fatty acid, PMR = partial mixed ration, a.p. = antepartum, p.p. = postpartum, GLC= gas liquid chromatography, GC = gas chromatography, FTIR= Fourier transform infrared-spectrometry, NEFA = non-esterified fatty acid, BHB = ß-hydroxybutyrate, DA = displaced abomasum

0.21, 0.4, 0.55 and 0.53, respectively. Assessed with data from literature, the R^2 values of one model with single-FA proportions and the four abovementioned ratio-based models were 0.75, 0.81, 0.85, 0.9 and 0.9, respectively, and the mean biases (MBs) were -153.8, 66.8, 48.7, 11.3 and -18.8 µmol/L, respectively. Overall, using the milk FA ratios C18:1 to C15:0 and C17:0 to C15:0 resulted in the best fits on both the internal and external data sets.

In Jorjong et al. [20], cis-9 C18:1 was the highest discriminating variable (R^2 = 0.38), followed by C16:0. Cross-validation results for grouping based on all variables resulted in an overall classification accuracy of 79.9 % with 80.3 % specificity and 75.0 % sensitivity, and cross-validation based on the most discriminating milk FAs only (i.e., cis-9 C18:1) showed an overall classification accuracy of 78.8 % with 79.1 % specificity and 75.0 % sensitivity.

In Mann et al. [9], none of the evaluated FAs in milk in the first week p.p. reached an AUC of ≥ 0.70. In milk samples from week 2 p.p., the FAs C15:0, cis-9 C16:1 and cis-9 C18:1 as well as the ratios cis-9 C18:1 to C15:0 and cis-9 C16:1 to C:15:0 yielded an AUC ≥ 0.70, with C15:0 at a threshold of ≤ 0.65 g/100 g being associated with the highest AUC in the analysis. Cis-9 C18:1 at a threshold of ≥ 24 g/100 g yielded the highest positive predictive value (76.1 %) but also the lowest negative predictive value (41.7 %). Cis-9 C16:1 and the ratio cis-9 C16:1 to C15:0 at thresholds of ≥ 1.85 g/100 g and ≥ 2.5 g/100 g had the highest accuracies of 70.7 % and 73.2 %, respectively, of all FAs and FA ratios for the correct classification of NEFAhigh.

Mantysaari et al. [21] found the highest correlation for the sum of C18:1 (r = 0.64 and r = 0.73 for morning and evening milkings, respectively) and for cis-9 C18:1 (r = 0.64 and r = 0.73). The model with the highest coefficient of determination of cross-validation (R^2cv = 0.63) used milk fat to protein ratio, change in body weight, DIM, C12:0, C14:0 and cis-9 C18:1 of the evening milking.

In Puppel et al. [22], significant differences in blood NEFA concentrations were found between cows with milk cis-9 C18:1 concentrations > 24 and those with ≤ 23.5 g/100 g fat. The mean values were 1.357 and 0.383 mmol/L NEFA, respectively. The mean value of the high cis-9 C18:1 group was above and the mean value of the low cis-9 C18:1 group was below the HYK threshold of 0.6 mmol/L.

The only significant finding in Puppel et al. [23] was a negative Pearson correlation coefficient of r = - 0.630 between the concentrations of n-6 C18:2 in milk and NEFA in blood in the second week p.p.

Comparison of milk FAs with blood BHB concentrations: Four studies [22-25] used plasma to determine the concentration of BHB, Nogalski et al. [26] used serum and Mann et al. [9] and Bach et al. [27] used full blood. In every study, a threshold concentration of 1.2 mmol/L BHB in blood was used as a cut-off value to distinguish HYK from non-hyperketonemic (nonHYK) cows, while Bach et al. [27] also included

Table 2: Changes in milk fatty acid (FA) and FA groups (FAs) concentrations and FA ratios for elevated non-esterified FA concentrations in blood (NEFA ≥ 0.6 [16, 20-23] or 1.0 [9] mmol/L, NEFAhigh) and hyperketonemia (BHB ≥ 1.2 mmol/L, HYK) found in the studies considered in this review

	FA/FAs/FA ratio	NEFAhigh	HYK
FA	C16:0	↑ (Jorjong et al., 2014 [20])	
	cis-9 C16:1	↑ (Mann et al., 2016 [9])	↑ (Mann et al., 2016 [9])
	C18:1 (*cis-9 C18:1, **trans-11 C18:1)	↑ (Mantysaari et al., 2019 [21]) *↑ (Jorjong et al., 2014 [20], Mantysaari et al., 2019 [21], Mann et al., 2016 [9], Puppel et al., 2017 [22])	*↑ (Mann et al., 2016 [9], Puppel et al., 2017 [22], Puppel et al., 2019 [23], Nogalski et al., 2015 [26], Van Haelst et al., 2008 [25]) **↓ (Nogalski et al., 2015 [26])
	n-6 C18:2		↓ (Puppel et al., 2019 [23])
	CLA (*cis-9,trans-11 C18:2, **trans-10,cis-12 C18:2)		↓ (Nogalski et al., 2015 [26]) *↓ (Puppel et al., 2019 [23]) **↓ (Puppel et al., 2019 [23])
	C20:5		↓ (Nogalski et al., 2015 [26])
FAs	C5:0 – C15:0 (*C7:0 – C13:0; **C15:0)	*↓ (Dórea et al., 2017 [16]) **↓ (Dórea et al., 2017 [16], Mann et al., 2016 [9])	↓ (Bach et al., 2019 [27]) **↓ (Mann et al., 2016 [9])
	C4:0 – C14:0 (*C4:0 – C8:0 + C12:0, **C6:0 – C14:0, ***C10:0 – C14:0)	**↓ (Dórea et al., 2017 [16]) ***↓ (Mantysaari et al., 2019 [21])	↓ (Bach et al., 2019 [27]) * ↓ (Puppel et al., 2019 [23]) **↓ (Mann et al., 2016 [9])
	n-6 FAs		↑ (Nogalski et al., 2015 [26])
	MCSFAs		↓ (Van Haelst et al., 2008 [25])
	LCFAs		↑ (Van Healst et al., 2008 [25])
	UFAs		↑ (Nogalski et al., 2015 [26])
FA ratios	cis-9 C16:1 to C15:0 ratio	↑ (Mann et al., 2016 [9])	↑ (Mann et al., 2016 [9])
	C17:0 to C15:0 ratio	↑ (Dórea et al., 2017 [16])	
	C18:1 to C14:0 ratio	↑ (Dórea et al., 2017 [16])	
	C18:1 to eSMCFAs ratio	↑ (Dórea et al., 2017 [16])	
	cis-9 C18:1 to C15:0 ratio	↑ (Dórea et al., 2017 [16], Mann et al., 2016 [9])	↑ (Jorjong et al., 2015 [24], Mann et al., 2016 [9])
	n-6 to n-3 FA ratio		↑ (Nogalski et al., 2015 [26])

FA = fatty acid, eSMCFAs = even short- and medium-chain FAs, MCSFAs = medium-chain saturated FAs, LCFAs = long-chain FAs, SFAs = saturated FAs, UFAs = unsaturated FAs, MUFAs = monounsaturated FAs, PUFAs = polyunsaturated FAs, CLA = conjugated linoleic acid

cows suffering from metritis and displaced abomasum (DA) that were culled or died, in contrast to healthy cows. Four studies [23-26] used a commercial kit on an analyzer to determine the BHB concentration, Mann et al. [9] and Bach et al. [27] used a cow-side handheld device, and one study [22] made no specification. Four studies [22, 23, 25, 27] collected milk and blood samples on the same day, while the remaining studies [9, 24, 26] collected blood samples over a longer period than milk samples and did not necessarily do so the same day. Bach et al. [27] used a fixed-effect multivariable Poisson regression and a ROC curve-based dichotomization as statistical methods. Jorjong et al. [24] and Van Haelst et al. [25] each used ANOVA as well as logistic regression and a nonparametric t-test, respectively. Nogalski et al. [26] used least-square analysis and Tukey's test. The statistical methods used in the remaining studies have been described earlier.

In Bach et al. [27], de novo FAs (C4:0 – C 15:0) were associated with an increased risk of disease or removal at all timepoints (T1 = 3 – 7 DIM, T2 = 6 – 11 DIM, T3 = 10 – 14 DIM, T4 = 13 – 18 DIM). Cut-off points were ≤ 22.7, ≤ 20.2, ≤21.0 and 21.1 g/100 g fat for T1, T2, T3 and T4, respectively, with sensitivities from 44.1 % (T2) to 61.5 % (T3 and T4) and specificities from 66.8 % (T1) to 83.1 % (T4).

In Jorjong et al. [24], the milk FA ratio cis-9 C18:1 to C:15:0 reached an overall classification accuracy of 75.2 %, a specificity of 78.5 %, a sensitivity of 75.3 %, and an R^2 value of 0.47 ($P < 0.001$). The threshold of the milk cis-9 C18:1 to C15:0 ratio associated with HYK decreased with time after parturition.

As for NEFAhigh in Mann et al. [9], none of the evaluated fatty acids in colostrum reached an AUC of ≥ 0.70 for the outcome of HYK. A total of eight fatty acids and two fatty acid ratios yielded an AUC ≥ 0.70 for HYK at week 2. At a threshold of ≤ 6.10 g/100 g, C14:0 reached the highest positive predictive value (92.9 %), and at a threshold of ≥ 54 g/100 g, the ratio cis-9 C18:1 to C15:0 reached the highest negative predictive value (90.4 %). Accuracy was highest (86.6 %) for a threshold of ≥ 3.76 g/100 g for the cis-9 C16:1 to C15:0 ratio.

In Nogalski et al. [26], the content of short-chain FAs (SCFAs) and medium-chain FAs (MCFAs) was significantly lower, and the content of LCFAs was significantly higher in the HYK group. Unsaturated FAs (UFAs) ($P < 0.01$) and n-6 FAs ($P \leq 0.05$) concentrations were also significantly higher and consequently, the n-6/n-3 fatty acid ratio was significantly higher ($P \leq 0.01$). Significant differences with lower concentrations in the HYK group were also found for vaccenic and eicosapentaenoic acid (no p-values given) and CLA ($P \leq 0.05$).

In Puppel et al. [22], significant differences in blood BHB concentrations were found between cows with milk cis-9 C18:1 concentrations > 24 and those with ≤ 23.5 g/100 g fat. The mean values were 1.103 and 0.753 mmol/L BHB, respectively. Both mean values were below the threshold ≥ 1.2 mmol/L BHB for distinguishing between HYK and nonHYK cows.

In Puppel et al. [23], the concentrations of C4:0; C6:0; C8:0; C12:0;

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Table 3: Identification method and most relevant milk fatty acids (FA), fatty acid groups (FAs) and FA ratios in predicting elevated non-esterified fatty acid concentrations in blood (NEFA ≥ 0.6 [16, 20-23] or 1.0 [9] mmol/L, NEFAhigh) and hyperketonemia (BHB ≥ 1.2 mmol/L, HYK) described in the studies considered in this review

	author, year	results (*thresholds (g/100 g for FA/FAs, g/g for FA ratios*) if specified)
NEFA high	Dórea et al., 2017 [16]	**AUC ≥ 0.80:** C6:0 (≤ 2.00), C7:0 (≤ 0.009), C8:0 (≤ 0.94), C9:0 (≤ 0.011), C10:0 (≤ 1.40), C11:0 (≤ 0.013), C12:0 (≤ 1.80), C13:0 (≤ 0.036), C14:0 (≤ 6.80), C15:0 (≤ 0.53), C17:0 to C15:0 (≥ 0.95), C18:1 to eSMCFAs ratio (≥ 2.60), C18:1 to C14:0 ratio (≥ 4.70), C18:1 to C15:0 ratio (≥ 62.00)
	Jorjong et al., 2014 [20]	**most discriminant variables (standardized canonical discriminant function coefficients):** cis-9 C18:1 (↑), C16:0 (↑)
	Mann et al., 2016 [9]	**AUC ≥ 0.70:** C15:0 (≤ 0.65), cis-9 C16:1(≥ 1.85), cis-9 C18:1 (≥ 26.00), cis-9 C18:1 to C15:0 ratio (≥ 45.00), cis-9 C16:1 to C:15:0 ratio (≥ 2.50)
	Mantysaari et al., 2019 [21]	**Pearson correlation coefficient:** C18:1 (↑), cis-9 C18:1 (↑)
	Puppel et al., 2017 [22]	**significant differences in mean values:** cis-9 C18:1 (> 24.00)
HYK	Bach et al., 2019 [27]	**Backward stepwise selection using a p > 0.05:** C4:0-C15:0 (≤ 22.70, ≤ 20.20, ≤ 21.00, ≤ 21.10 for 3 – 7, 6 – 11, 10 – 14 and 13 – 18 DIM, respectively)
	Jorjong et al., 2015 [24]	**most discriminant ratio:** cis 9 C18:1 to C15:0 ratio (↑)
	Mann et al., 2016 [9]	**AUC ≥ 0.70:** C6:0 (≤ 1.68), C8:0 (≤ 0.80), C10:0 (≤ 1.60), C12:0 (≤ 1.42), C14:0 (≤ 6.10), C15:0 (≤ 0.50), cis-9 C16:1 (≥ 1.83), cis-9 C18:1 (≥ 30.00), cis-9 C18:1 to C15:0 ratio (≥ 54.00), cis-9 C16:1 to C15:0 ratio (≥ 3.76)
	Nogalaski et al., 2015 [26]	**significant differences in mean values:** UFAs (↑), n-6 FAs (↑), n-6/n-3 FA (↑), cis-9 C18:1 (↑), trans-11 C18:1 (↓), CLA (↓), C20:5 (↓)
	Puppel et al., 2019 [23]	**Multivariate analysis:** n-6 C18:2 (↓), cis-9,trans-11 C18:2 (↓), trans-10,cis-12 C18:2 (↓)
	Van Haelst et al., 2008 [25]	**significant differences in mean values:** LCFAs (↑), cis-9 C18:1 (↑) **tendency in mean values:** MCSFA (↓)

AUC = area under the receiver operating characteristic curve, eSMCFAs = even short- and medium-chain FAs, CLA = conjugated linoleic acid, UFAs = unsaturated fatty acids, FA = fatty acid, LCFAs = long-chain FAs, MCSFAs = medium-chain saturated FAs, MUFAs = monounsaturated FAs, PUFAs = polyunsaturated FAs, SFAs = saturated FAs

cis-9,trans-11 C18:2 and trans-10,cis-12 C18:2 were significantly decreased for HYK cows in the first and second week of lactation, and the concentrations of cis-9 C18:1 were significantly increased for HYK cows in the first and second week of lactation. The concentrations of all n-6 C18:2 were significantly decreased in the second week of lactation. A significant correlation was found for BHB and cis-9,trans-11 C18:2 (r = -0.732 and r = -0.520 in week 1 and 2, respectively) as well as for BHB and trans-10,cis-12 C18:2 (r = -0.821 and r = -0.635).

Van Haelst et al. [25] found a tendency for greater LCFAs proportions in HYK cows. Significantly greater milk LCFAs and lower medium-chain saturated FAs proportions were measured at the week of diagnosis only. Cis-9 C18:1 concentrations in milk fat (g/100 g) were 3.46, 4.42, and 2.08 units greater in HYK cows in the prediagnosis, diagnosis, and postdiagnosis periods, respectively. Elevated proportions of cis-9 C18:1 were detected in milk fat two weeks before the HYK diagnosis, making it an interesting trait for subclinical ketosis prediction.

Discussion

Methodological aspects: The greatest challenges when comparing different studies are the varying study designs. Apart from one study, all experiments were conducted on HF cows. Reproducibility within one breed was high, but FA concentrations also showed similar correlations with the reference in both HF and NR cows. Suggested thresholds or prediction models should be validated for each breed.

Varying management practices: Cows in some studies were subject to additional research exceeding the subject of this review. In some groups, various feeding or dry management protocols were performed. This led to less comparability between studies, as it was shown that the FA composition of bulk tank milk is influenced by management practices and dietary composition [28, 29] and reflects more realistically the vast spectrum of influencing factors. Bulk tank milk composition or management practices including dietary composition should be included in prediction models. The studies used either morning, evening, morning and evening or pooled milking samples. There is evidence that NEFA concentration is better predicted from evening than from morning milk samples [21]. As milk composition varies slightly between morning and evening milkings [30], this should be taken into account when working with described threshold concentrations of depicted milk FAs.

Comparability of the references: Eight studies used GC as a standard method to determine the concentration of the FAs. When using GC, it is important to recognize that concentrations of FAs contained in a large proportion, such as LCFAs, are determined more reliably than FAs contained in smaller proportions, such as SCFAs [16, 31]. Two studies used FTIR to determine the concentrations of the FAs. When using FTIR, smaller proportions of FAs are also not determined as precisely, whereas larger proportions can be predicted with greater accuracy [16, 28, 32, 33]. Poor prediction might limit the use of FTIR for determining FA profiles in milk [16]. For NEFA concentration, Mann et al. [9] used a different NEFA threshold (1.0 mmol/L) than the other studies (0.6 mmol/L) to prevent overestimating slightly elevated concentrations that might occur within increased sampling frequency. This should not affect the general validity of the detected FAs, as they would still have a possible use in predicting metabolic status, but the different NEFA threshold should again be considered when working with suggested threshold concentrations.

Another question raised is which of the references, BHB or NEFA, is best associated with metabolic diseases. Epidemiological data have established the association between increased BHB and NEFA concentrations in blood as indicators of a metabolic disorder and of negative health, production and reproduction outcomes at both the individual cow and herd level [4, 8, 9]. Blood BHB concentration has been used as the gold standard in diagnosing ketosis for many years now [1, 2]. In a more recent study, Tremblay et al. [5] demonstrated that blood NEFA concentrations were most significantly correlated with PMAS classes.

According to Gonzalez et al. [34], NEFA is a more reliable indicator for lipolysis than the milk fat to protein ratio. Tremblay et al. [35] also found that milk FA profiles are more useful for predicting NEFA than BHB. By including negative health outcomes in the HYK group, Bach et al. [27] used an approach that seems more meaningful to define certain milk FA profiles associated with negative outcomes instead of using other metabolites that have limitations. Large epidemiological studies are needed to establish the association of certain milk FA profiles and negative health and production outcomes at both the herd and cow level.

Sampling timing and frequency: Some studies took milk samples at the same time as the reference (blood sample), while this was not the case in other studies. Simultaneous collection of milk and blood samples is of course the most precise method. Especially as an early warning for a possibly deteriorating metabolic status, it might be useful to compare milk samples with blood samples taken at a later time, though. Van Haelst et al. [25] considered the difference in collecting milk fatty acids before and after the reference in cows diagnosed with hyperketonemia (HYK). Although the results were not significant, trends regarding different FA profiles between groups (HYK and nonHYK) were observed while there was no difference in blood BHB concentration, indicating that the FA profile changes before the BHB concentration changes. All studies evaluated different numbers of blood and milk samples. The predictive accuracy is likely increased if a larger number of samples is taken.

Statistical methods: Most studies focused on finding one milk FA concentration, FA group or FA ratio correlated to an unfavorable metabolic status. One hypothesis is that correct classification and sensitivity can be increased by a combined testing with various FA concentrations/FA ratios or whole FA profiles included in prediction models. Jorjong et al. [20] found that a classification based on one FA was only slightly less specific than one based on the full parameter set. In Dorea et al. [16], the model using a larger number of FAs after the elimination of FAs showing collinearity had a better root-mean-square error and Akaike information criterion than the model from which a few FAs were excluded to fit an external data set. One example of collinearity is that the majority of unsaturated fatty acids (UFAs) are LCFAs, which is why Nogalski et al. [26] noted significantly higher ($P < 0.01$) UFAs concentrations in the HYK group. In Mantysaari et al. [21], the model assessed with the highest coefficient of determination of cross-validation used the milk fat to protein ratio, change in body weight, DIM, and the C12:0, C14:0 and cis-9 C18:1 FAs from the evening milking, indicating that including additional information aside from FA concentration might further improve prediction accuracy.

Biological aspects: As milk FAs originate from the four major sources of diet, de novo synthesis in the mammary gland, formation in the rumen by biohydrogenation or bacterial degradation and release from body fat stores [36, 37], changes in milk-fat-composition, both over lactation and during metabolic disorder, imply shifts in the activity of these pathways and are related to changes in the energy status of the cow [36, 38, 39]. Diet composition has a great influence on the milk FA profile and should therefore be taken into consideration when interpreting predictions made on the basis of milk FAs [16], as mentioned earlier. When comparing bulk tank milk from different farms, there is evidence that management practices, such as overcrowded free stalls and reduced feeding frequency, as well as dietary components, for instance greater dietary ether extract and lower physically effective neutral detergent fiber content, are associated with lower de novo FA synthesis [28, 29]. Overall, there was high agreement among the studies examined regarding the changes in the FA profile both within and between different references. Decreased short- and medium-chain FA (C4 – C14 and C5 – C15) concentrations were associated with metabolic disorders [9, 16, 21, 23, 25, 27]. They are derived from de novo synthesis from acetate and, to a lesser extent, from butyrate [18], which is reduced during energy shortage. An elevated concentration of cis-9 C18:1 during increased NEFA or BHB concentration has been reported and discussed by several authors [9, 20, 25, 26]. As a predominant FA in ruminant adipose tissue [40], cis-9 C18:1 reflects the influence of body fat mobilization on the FA profile and therefore is highly correlated with metabolic disorders [9, 16, 19]. Furthermore, the cis-9 C18:1 to C15:0 ratio is also described as having potential in diagnosing the metabolic health status by various authors [9, 16, 24]. Containing both an FA derived from body fat mobilization and one from de novo synthesis, this value combines two characteristics within one ratio.

Economical aspects: Jorjong et al. [20] addressed the economic effect of using milk FAs to predict ketosis and claimed that cow-side tests that allow the selective treatment of cows at risk would only be used routinely when the cost of such tests does not exceed potential gain. There is evidence suggesting that a test and treat approach is a profitable strategy [11]. Additionally, the economic benefit strongly depends on the incidence rate [20]. With a high incidence rate of metabolic problems, the most cost-effective solution might be to treat all animals, whereas the opposite is true when the incidence rate is low. Based on the cost effectiveness simulation used in the study, a maximum gain of approximately 2 € per case was calculated for the early warning of detrimental blood NEFA based only on cis-9 C18:1, not including the costs for milk FA analysis.

Refinement of predictions and future aspects: FA profiles in the blood differ between healthy cows and cows with uterine infections p.p. or reduced fertility [41], leading to the assumption that FA profiles in the blood are associated with reproductive processes. This is likely to be reflected by different FA profiles in the milk, as well, which possibly extends the use of milk FA profiles, as also shown by Bach et al. [27], who also covered other diseases in the HYK group.

It seems to be difficult to manifest a certain threshold for one or two FA concentrations or ratios in predicting metabolic diseases [20, 24]. To further refine the prediction of the metabolic status, FA profiles both between and within herds could be compared and taken as a reference when predicting the status for an individual cow. After all, it has been shown that bulk tank milk samples from different herds have different FA profiles depending on management factors such as feeding frequency, stocking density and body condition [9, 28]. Including lactational stage as it affects daily milk yield, milk composition and FA profile [25, 42, 43], milk yield as it in turn modifies the FA profile [25, 36], and the number of lactations into prediction models might further improve the accuracy of the predictions. Dorea et al. [16] discuss that poor predictions might limit the use of FTIR in determining FA profiles in milk. On the other hand, FTIR, as a high-throughput technology, is already implemented as a routine analysis and might therefore be a promising tool in the assessment of the metabolic status of a cow and the whole lactating herd if models become more precise by including influencing factors and increased sample sizes [35, 44-46]. As experiments have been mostly conducted on HF cows, further studies on HF cows and other breeds should aim to establish models predicting metabolic disorders using milk FA concentrations and other influencing factors.

Conclusions

A few single fatty acid concentrations, such as those of cis-9 C16:1, as well as fatty acid ratios, such as cis-9 C16:1 to C15:0, C17:0 to C15:0 and

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C18:1 to C15:0, are correlated with elevated blood ß-hydroxybutyrate or non-esterified fatty acid concentrations. Some might be useful in routine herd health monitoring despite having only moderate correlation coefficients. Implementing measuring milk fatty acid profiles in routine herd health monitoring becomes even more interesting with using Fourier transform infrared spectroscopy techniques, as they are easy, fast and cost-effective. The implementation of refined prediction models that use all available information to predict the health status of both individual cows as well as the whole herd as exactly as possible may be more promising than the use of single fatty acids or fatty acid ratios to detect cows suffering from metabolic disorders. Future studies should address further improvements of prediction models by enlarging sample sizes and refining the models by including influencing factors (e.g. number of lactations, season, energy balance average of the herd, milk yield, dietary composition and days in milk). Based on the findings of already existing and future large epidemiological studies, refined prediction models are predicted to become a supporting tool in routine herd health monitoring.

Compliance with Ethical Standards

The authors declare no conflict of interest.

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