Examinations on the Metabolic Monitoring Using Blood and Milk Fatty Acid Profiles in Simmental Cows After Parturition

von Anne Marlies Reus

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von Anne Marlies Reus

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"I know that I know nothing."

Sokrates

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ABBREVIATIONS

AMS	automatic milking systems
BCS	body condition score
BHBA	ß-hydroxybutyrate acid
CLA	conjugated linolenic acid
C16:0	palmitic acid
C18:0	stearic acid
C18:1	oleic acid
DIM	days in milk
FA	fatty acid
FEQ	Fett-Eiweiß-Quotient
FPR	milk fat-to-protein ratio
FTIR	Fourier-transform infrared
NEB	negative energy balance
NEFA	non-esterified fatty acids
PMAS	Poor Metabolic Adaptation Syndrome
RMSE	root mean squared error
SCC	somatic cell count
SCFA	short-chained fatty acids
SCK	subclinical ketosis
SD	standard deviation

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I. INTRODUCTION

Production animals worldwide have become increasingly productive over the last few decades, mainly through breeding and management improvements (Rauw et al., 1998; Hansen, 2000; Norring et al., 2012). Dairy cows are no exception; on the contrary, the average cow's milk yield within the European Unit increased from 5,585 kg in 2001 (Marquer, 2013) to 7,682 kg in 2019 (Anonym., 2022), indicating an increase of 37.5% within 20 years. Increased productivity has a lot of benefits, such as a smaller number of animals for the same production yield (Hansen, 2000), resulting in less time and resources needed, leading to less emissions and waste produced for the same output. On the other hand, intensified productivity poses challenges as well. For example, increased time investment in management is required, the appearance of new production diseases can occur, and reduced cow longevity can occur (Rauw et al., 1998; Hansen, 2000).

Higher productivity implies that the milk yield moves towards the physiological boundaries of the cows. Breeding efforts continue to improve productivity and overcome what were considered physiological boundaries (Hansen, 2000). Cows can produce high milk yields if all needs, such as housing environment and nutritional needs, are adequately met, and many factors have to be adjusted optimally (Schrader, 2009). Otherwise, either productivity decreases or – if the cow is genetically determined to continue with a high milk yield – animal welfare decreases, and the cow’s health decreases (Schrader, 2009).

Metabolic imbalances are one major challenge (Schrader, 2009). Abundant research was carried out on ketosis as the most prominent metabolic imbalance and defined disorder. Prevalence rates for subclinical ketosis from 8 to 22% in the first two months of lactation are described (Duffield, 2000; Suthar et al., 2013; Tatone et al., 2017). The incidence of subclinical ketosis ranges from 16 to 43%, depending on the lactation number (McArt et al., 2012; Gordon et al., 2013). Subclinical ketosis can become clinical by showing a reduction in milk production, feed intake, or foregut motility, as well as a loss of bodyweight, dry and dark feces, or unwillingness to move. Neurological signs such as aggressiveness, nervousness, trembling, or roaring are also described (Baird, 1982; Berge and Vertenten, 2014; Gruber and Mansfeld, 2019). Subclinical ketosis is associated with other production diseases like metritis, clinical ketosis, displaced abomasum, lameness, placental retention, and culling within 60 days postpartum (Suthar et al., 2013; Raboisson et al., 2014; Abdelli et al., 2017; Gruber and Mansfeld, 2019). For a long time, the quantitative analysis of ß-hydroxybutyrate acid (BHBA) in blood was considered the gold

standard in diagnosing ketosis (Duffield et al., 1997; Oetzel, 2004). Recent research suggests that the concentration of non-esterified fatty acids (NEFA) in blood indicates more reliably the extent of a metabolic disorder (McArt et al., 2013; Tremblay et al., 2018).

The aim of this work was to investigate the possibility of reliably diagnosing and predicting the occurrence of a metabolic disorder by comparing milk and blood fatty acid (FA) concentrations of healthy cows to those of cows suffering from metabolic imbalances using linear regression models. Both milk and blood FA concentrations were determined by using milk FTIR data available from the high-throughput routine milk analysis.

Animal welfare can be high in human husbandry, and cows can be adequately kept in a stable (Schrader, 2009; Andreasen et al., 2020). If managed well, they receive an appropriate quality and amount of food and care, receive treatment if injured or ill, and live in an animal-friendly husbandry. Optimal treatment in all circumstances without mismanagement, neglect, or mistreatment seems a high goal to achieve (Andreasen et al., 2020). Nevertheless, we should strive for this aim, and many steps will lead to many minor and major improvements. This thesis will potentially and hopefully lead to an improvement in the management of dairy herds.

II. LITERATURE OVERVIEW

1. Ketosis

1.1. Definition

The term ketosis is derived from the ketone bodies and describes elevated blood concentrations of the same. Ketone bodies are acetoacetate, acetone and ß-hydroxybutyrate acid (BHBA). As BHBA concentrations are easiest to determine, the BHBA concentration in blood is generally used as a reference to describe hyperketonemia. The quantitative analysis of BHBA was and is still considered to be the gold standard in the diagnosis of ketosis (Duffield et al., 1997; Oetzel, 2004). The term ketosis was traditionally used for cows showing clinical signs with a concurring hyperketonemia. It is well established in recent decades to distinguish between clinical and subclinical ketosis. Subclinical ketosis (SCK) is defined by serum BHBA levels > 1.0 to 1.4 mmol/L without clinical signs of ketosis (Duffield, 2000; Iwersen et al., 2009; Suthar et al., 2013). Clinical signs start at about > 2.6 mmol/L, while the threshold is extremely variable at an individual cow level (Andersson, 1984; Duffield, 2000).

1.2. Prevalence and Incidence

Prevalence rates for subclinical ketosis ranging from 8 – 22% in the first two months of lactation are described (Duffield, 2000; Suthar et al., 2013; Tatone et al., 2017; Gruber and Mansfeld, 2019). The incidence for subclinical ketosis ranges from 16 – 43%, depending on the number of lactation (McArt et al., 2012; Gordon et al., 2013; Gruber and Mansfeld, 2019).

1.3. Symptoms

The clinical signs showed by cows during a ketosis can be a reduction in milk production, feed intake or foregut motility, as well as a loss of bodyweight. Dry and dark faeces may also be present or an unwillingness to move. Furthermore, neurological signs as aggressiveness, nervousness, trembling or roaring can appear (Baird, 1982; Berge and Vertenten, 2014; Gruber and Mansfeld, 2019).

1.4. Associated Diseases

Subclinical ketosis is associated with other production diseases like metritis, clinical ketosis, displaced abomasum, lameness, placental retention and culling within 60 days postpartum (Suthar et al., 2013; Raboisson et al., 2014; Abdelli et al., 2017; Gruber and Mansfeld, 2019).

1.5. BHBA Concentrations and Clinical Appearance

Even though ketosis is named after ketone bodies and describes a disease that is associated with hyperketonemia, elevated blood ketone levels do not manifest consistently with the clinical signs (Andersson, 1984; Duffield et al., 2009; Tremblay et al., 2018). Cows showing clinical signs can have low or intermediate blood BHBA levels, while cows with high blood BHBA levels do not necessarily show clinical signs. As a result, Tremblay et al. (2018) introduced the term "pour metabolic adaptation syndrome (PMAS)".

2. Pour Metabolic Adaptation Syndrome (PMAS)

It is reasoned that the current gold standard in diagnosing cows suffering from ketosis, the detection of hyperketonemia (blood BHBA $\geq$ 1.2 mmol/L), does not consistently manifest with clinical symptoms of ketosis or indications of poor metabolic adaptation during early lactation (Andersson, 1984; Duffield et al., 2009; Tremblay et al., 2018). Physiologically, high-yielding milking cows enter a phase of negative energy balance (NEB) after parturition due to high energy demands of milk production and a dry matter intake that cannot match the energy requirements (Baird, 1982; Bell, 1995). It is suggested that cows can either compensate for NEB by reducing fat in milk or by increasing fat mobilization from adipose tissue and that only cows increasing fat mobilization persistently developed hyperketonemia (Klein et al., 2012; Tremblay et al., 2018). Cows with poor metabolic adaptation are characterized by an inappropriate reaction to the negative energy balance in early lactation (Baird, 1982; Tremblay et al., 2018). Indications of PMAS are expected to be elevated liver enzymes and bilirubin, decreased rumen fill, reduced rumen contractions, and a decrease in milk production (Ghanem et al., 2016; Issi et al., 2016; Cao et al., 2017; Tremblay et al., 2018). In general, higher producing, older cows being in early lactation with a higher body condition score before parturition are more often affected by PMAS (Baird, 1982; Rukkwamsuk et al., 1999; Ghanem et al., 2016; Tremblay et al., 2018). Metabolic diseases, unlike infectious diseases, cannot easily be defined as diseased or not diseased, they are rather defined as syndromes observed on a

spectrum of signs (Tremblay et al., 2018). Therefore Tremblay et al. (2018) addressed the problem of differentiating classes of PMAS and defined three classes: low, intermediate and high PMAS, which did not follow differences in BHBA levels. The argument is brought forward, that this may be a consequence of the fact, that ketogenesis and resulting ketonemia are normal physiological responses to compensate for NEB as mentioned above and do not necessarily reflect pathological changes (Tremblay et al., 2018). This leads to the conclusion, that it is important to be able to distinguish between appropriate and inappropriate responses to NEB (Tremblay et al., 2018). In accordance with Klein et al. (2012), Tremblay et al. (2018) were able to distinguish within the group of cows suffering from intermediate PMAS, between cows increasing ketogenesis (indicated by hyperketonemia) and cows limiting milk fat (indicated by a reduced milk fat). A cause for limited milk fat can be limited ketogenesis (Baumgard et al., 2000; Tremblay et al., 2018). Cows showing the highest agreement with expected PMAS indicators did not decrease milk fat or increase ketogenesis, suggesting that they did not adapt appropriately (Tremblay et al., 2018). Furthermore, it is suggested that PMAS classes can be identified by NEFA cut-off values of <0.39 mmol/L (95% CI: 0.360–0.410) for low PMAS observations and ≥0.7 mmol/L (95% CI: 0.650–0.775) for high PMAS observations, as they consistently characterize PMAS classes (Tremblay et al., 2018).

3. Metabolic Diseases and Herd Health Management

3.1. Definition

Unlike decades ago, where veterinarians treated mainly individual animals, integrated herd health management nowadays concentrates on the prevention of diseases and the performance of the dairy herd (de Kruif and Opsomer, 2004). This requires excellent housing facilities as well as a functioning cooperation between veterinarians and farmers or managers. The prevention of diseases as a main management aim relies strongly on an integrated herd health management. Herd health management improves both animal health and welfare and helps to maintain a high quality of foods from animal origin (de Kruif and Opsomer, 2004). The administration of medication remains necessary but must be carried out under strictly controlled conditions (de Kruif and Opsomer, 2004).

3.2. The Role of Metabolic Diseases in Herd Health Management

The following factors describe the importance of a disease regarding herd health management: occurrence of the disease (prevalence and incidence) as well as the severity which is described by the symptoms, associated diseases and associated costs. With an average herd prevalence of 21%, and an average incidence of approximately 40% within the first 2 weeks after calving, subclinical ketosis is a relevant factor in herd health monitoring (Gruber and Mansfeld, 2019). Concerning the second factor, the main aspect during a subclinical ketosis is, although not showing clinical signs, the milk yield decrease in the first 2 weeks postpartum by 3 – 5.3 kg/d for each ketotic cow, and the total average milk reduction through the whole lactation period of 305 days by 112 kg (standard deviation (SD): 89 kg) (Gruber and Mansfeld, 2019). Furthermore, during a subclinical ketosis the risk of developing associated production diseases like retained placenta, metritis, displaced abomasum, lameness and clinical ketosis increases, while the herd health status deteriorates and the risk for early culling increases (Gruber and Mansfeld, 2019). The financial aspect includes costs for early death, reduced milk production, reproduction losses and associated production diseases. The calculated costs per case of subclinical ketosis vary between $ 78 and $ 289 (Gruber and Mansfeld, 2019).

3.3. Current Monitoring Strategies

Metabolic disorders are tightly linked to the physiologically occurring negative energy balance after calving. Therefore, monitoring the NEB after calving can help to monitor and predict metabolic disorders (Roche et al., 2013; Gruber and Mansfeld, 2019). Concurring with a NEB the cow loses body weight. This can be rated by using the body condition score (Edmonson et al., 1989). The suggested body condition score (BCS) ranges from 1 to 5, using .25-unit increments. A score of 1 indicates an emaciated condition, and a score of 5 indicates an obese condition (Edmonson et al., 1989). Eight body characteristics are used to evaluate the BCS to obtain a maximum of objectivity throughout the evaluation of various persons using the BCS (Edmonson et al., 1989). If the cow loses excessive body weight during the first weeks after parturition, it can be concluded that she underwent a severe NEB. This suggests that she suffered severe metabolic challenges and has a higher risk of a metabolic disease. Consistently, not the actual BCS but rather the change of BCS can be used to monitor the risk factor of a severe NEB (Gruber and Mansfeld, 2019). This method is easy and cost-effective, yet it requires discipline and is work intensive to evaluate the cows at risk regularly (Gruber and Mansfeld, 2019). Furthermore, even though it objectifies the evaluation by using eight body

characteristics, the human factor remains and it is error-prone due to possibly occurring operational blindness. Another disadvantage is, that if severe body weight loss has taken place and gets noticed, a lot of metabolic challenge has already happened and the cow may already be in a positive energy balance again (Bünemann et al., 2019). Furthermore, it is suggested, that ultrasonic measurements considering inner fat depots is more accurate (Bünemann et al., 2019).

Another possibility to monitor metabolic diseases are cow side ketosis tests. A few commercial tests are available. They can be conducted on milk, urine or blood (Gruber and Mansfeld, 2019). Taking blood requires a veterinarian, while urine and milk can be sampled by the farmer. Milk samples are easy to obtain and testing the sample only takes a small amount of time. While specificities of 96 and 97% for a cow side test using milk are reported for a serum BHBA threshold of 1.2 and 1.4 µmol/L, respectively, sensitivities showed only 88 and 96%, respectively (Iwersen et al., 2009). Collecting urine samples is more complicated and while the sensitivity was 100%, the specificity was only 59% (Nielen et al., 1994). Regarding the gain of information, cow side milk tests can be very useful. They can be of valuable help in testing suspicious animals. If conducted on the whole herd or even on all cows during the period at risk after calving, it can, though, amount to be work and cost intensive. As a consequence, they cannot be considered to be an effective means in monitoring the whole herd regarding metabolic disorders (Gruber and Mansfeld, 2019).

Additional to the information farmers can obtain from automatic milking systems (AMS) or other automated monitoring features, the federal states in Germany offer monthly (11 per year) analysis of every single cow's milk. A sample is collected from one milking and the milk yield is determined (Anonym.). Milk fat, milk protein, milk urea and milk lactose concentration as well as the somatic cell count (SCC) are determined based on high throughput Fourier transform infrared spectroscopy and edited (Anonym., 2019). Mainly helpful in determining cows at risk are milk fat and milk protein as well as the milk fat-to-protein ratio, as they reflect the energy and protein intake in relation to the cow's need (Garcia et al., 2015). The cow's demand is created by her metabolic turnover as well as her milk production. This method can be very helpful for the monitoring of metabolic diseases (Garcia et al., 2015). On the downside, evaluating the results of the routine milk analysis is often underrated and neglected and therefore a great potential is lost. The potential is further limited by the only monthly measurement. In the worst case, the first analysis after calving takes place only a month after calving if the previous analysis took place just before calving. The cow passes the period with the highest risk without receiving an analysis of her milk and therefore without being monitored (Gruber et al., 2021).

Some states offer early warnings for metabolic stress or ketosis additional to the established monthly analysis. For example, recent research established a so-called "double traffic light" in Bavaria (Anonym., 2019). One traffic light indicates the risk for an increase of fat mobilization by predicting the blood NEFA concentration using the Fourier-transform infrared (FTIR) spectra data. As a traffic light, it has three levels: green – low risk for metabolic stress, yellow – intermediate risk and red – high risk. The second traffic light predicts the amount of ketone bodies in the blood and uses this information in combination with the milk fat-to-protein ratio to predict the risk for ketosis again in three levels: green – low risk for ketosis, yellow – intermediate risk and red – high risk (Anonym., 2019). Similar predictions have been established in other states and countries, e.g. KetoMIR in Baden-Württemberg (Drössler et al., 2018).

4. Relevant Fatty Acids in Blood and Milk in the Context of Metabolic Herd Health Monitoring

4.1. Physiologically Occurring Fatty Acids in Blood and Milk

4.1.1. Physiologically Occurring Fatty Acids in Blood

The Qlip N.V. (Leusden, The Netherlands) models for FA in blood differentiate between 55 FA (Table 1). C18:1, C18:0 and C16:0 are the FA with the highest concentration.

Table 1: Qlip N.V. (Leusden, The Netherlands) mean fatty acid concentrations in blood in µmol/L from n=3 farms in the Netherlands.

Fatty acid	Concentration in µmol/L (standard deviation)
C13:0	0.15 (0.04)
C14:0	4.60 (2.90)
C14:1	0.96 (0.97)
C15:0	3.68 (1.66)
C16:0	66.44 (45.79)
C16:1	9.18 (8.37)
C16:2	0.18 (0.09)
C17:0	8.56 (5.65)
C17:1	4.03 (2.54)
C18:0	103.93 (45.47)
C18:1	113.86 (88.02)
C18:2	14.33 (6.46)
C18:3	4.46 (2.32)
C18:4	0.10 (0.04)
C19:0	1.01 (0.55)
C19:1	1.69 (0.83)
C19:2	0.35 (0.12)
C19:3	0.07 (0.02)
C20:0	1.39 (0.44)
C20:1	1.07 (0.68)
C20:2	0.51 (0.14)
C20:3	1.27 (0.32)
C20:4	3.14 (1.25)
C20:5	0.90 (0.37)
C21:0	0.37 (0.08)
C21:1	0.16 (0.06)
C21:3	0.09 (0.01)
C21:4	0.18 (0.02)
C22:0	1.20 (0.31)
C22:1	0.41 (0.08)
C22:3	0.11 (0.02)
C22:4	0.29 (0.08)
C22:5	1.41 (0.49)
C22:6	0.18 (0.05)
C23:0	1.04 (0.30)
C23:1	1.44 (0.43)
C24:0	2.28 (0.66)
C24:5	0.07 (0.02)
C24:6	0.18 (0.23)
C25:0	0.49 (0.10)
C25:1	0.58 (0.13)
C25:3	0.09 (0.03)
C25:5	0.05 (0.01)
C26:0	5.52 (1.06)
C26:1	0.20 (0.03)
C26:2	0.05 (0.01)
C27:0	0.13 (0.03)
C27:1	0.07 (0.02)
C27:3	0.09 (0.03)
C28:0	0.59 (0.16)
C28:1	0.06 (0.02)
C29:0	0.07 (0.02)
C29:4	0.25 (0.07)
C29:6	0.20 (0.03)
C30:1	0.06 (0.01)

4.1.2. Physiologically Occurring Fatty Acids in Milk

In general, milk fat composition is largely influenced by feed intake (Bauman and Griinari, 2003). Ruminants are thus an exception, as milk fat composition is largely influenced by bacterial metabolism in the rumen (Bauman and Griinari, 2003). Milk fat from ruminants is expected to contain more than 400 different FA which relates mostly to the bacterial metabolism in the rumen (Bauman and Griinari, 2003). The physiological milk fat composition of cows is described in Bauman and Griinari (2003) as the following in molar percent: C4:0: 12, C6:0: 5, C8:0: 2 C10:0: 4, C12:0: 4, C14:0: 11, C16:0: 24, C16:1: 3 C18:0: 7, C18:1: 24, C18:2: 3, C18:3: 1, >C18:3: <1 (Jensen, 2002). Dorea et al. (2017) describe the following FA as relevant in the milk: C4:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C17:0, C18:0 and C18:1. Partially overlapping with those FA are the FA examined in Mann et al. (2016): C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, cis-9 C14:1, C15:0, C16:0, cis-9 C16:1, C17:0, C18:0, trans-9 C18:1, trans-10 C18:1, trans-11 C18:1, trans-12 C18:1, cis-9 C18:1, cis-11 C18:1, cis-12 C18:1, cis-9,cis-12 C18:2, cis-9,trans-11 C18:2 and cis-9,cis-12,cis-15 C18:3. Another study considered C4:0, C10:0, C12:0, C14:0, C16:0, C18:1 as well as the sums of cis-9 C18:1 and cis C18:1 as relevant (Mantysaari et al., 2019). In a further study the following FA are called functional: C4:0, C18:1 trans-11, C18:1 cis-9, C18:2, C18:2 cis-9,trans-11, C18:3, C 20:4, C 20:5 and C22:6 (Nogalski et al., 2015).

4.2. Changes in the Fatty Acid Composition during Ketosis/PMAS

After calving and during the beginning of lactation, cows enter a period of a NEB, meaning that the extent of energy in form of fat that is used for milk production exceeds the energy intake by feed (Baird, 1982). It is suggested that cows can react to the NEB with one of the following strategies, either by reducing fat in the milk or by increasing fat mobilization from adipose tissue (Klein et al., 2012; Tremblay et al., 2018). Both strategies naturally have an impact on the milk fat composition. Furthermore, they also affect the blood fat composition, as fat mobilized from adipose tissue directed to the milk is transported via the blood stream and fat not used for milk production remains in the blood until further metabolization (Bauman and Griinari, 2003).

4.2.1 Changes in the Blood Fatty Acid Composition during Ketosis/PMAS

A lot of research has been done on the concentration of NEFA in blood during metabolic disorders (Jorjong et al., 2014; Mann et al., 2016; Dorea et al., 2017; Puppel et al., 2017; Mantysaari et al., 2019). Other than NEFA, FA concentrations in the blood have not been the subject of extended research. Little is known on the changes in the FA composition in the blood during a metabolic disorder.

4.2.2 Changes in the Milk Fatty Acid Composition during Ketosis/PMAS

Milk FA are derived from two sources: intake from the blood and de novo synthesis in the mammary gland (Bauman and Griinari, 2003). Short-chained (4-8C) and medium-chained (10-14C) FA are almost always derived by de novo synthesized FA, long-chained (>16C) FA are derived from blood circulation and FA containing 16Cs are derived from both. By determining the milk FA profile, the source of the FA can be derived, which is further explained in the following (Bauman and Griinari, 2003): In contrast to non-ruminants using glucose, ruminants use acetate as a source of carbohydrate for FA synthesis. Acetate originates from ruminal fermentation of carbohydrates. BHBA provides about half of the first 4 Cs in de novo synthesized FA (Bauman and Griinari, 2003). Physiologically, lipolysis and metabolization of body fat forms less than 10% of the FA in milk. During a state of negative energy balance, the ratio of mobilized fat increases directly proportional to the extent of energy deficiency. As a consequence, the amount of long-chained and unsaturated FA increases while short- and medium-chained FA decrease (Bauman and Griinari, 2003).

4.3. Previous Research and Use of the Fatty Acid Composition in Blood and Milk

The first publication included in this thesis (Reus and Mansfeld, 2020) gives an overview of the research on the use of milk FA composition for the prediction of metabolic health status.

III. PUBLICATIONS

1. Publication 1

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

Anne Marlies Reus, Rolf Mansfeld

Clinic for Ruminants with Ambulatory and Herd Health Services

Centre of Clinical Veterinary Medicine of the Ludwig-Maximilians-University Munich

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

Anne Marlies Reus[1], Rolf Mansfeld[1]

[1]Ludwig-Maximilian-University Munich, Clinic for Ruminants with Ambulatory and Herd Health Services, Sonnenstr. 16, 85764 Oberschleissheim (LMU Munich)

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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

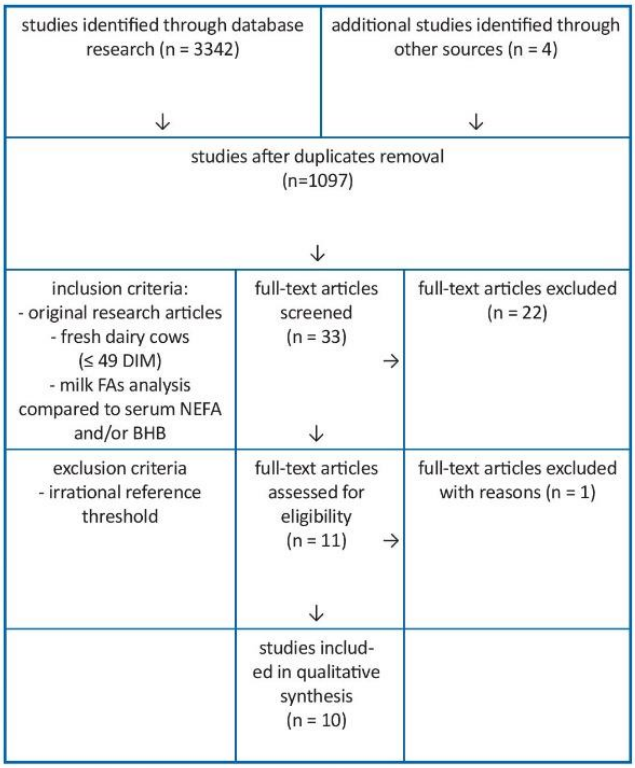

Figure 1: Flow diagram describing the review process

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;

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

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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2. Publication 2

Differentiating Between Metabolic Health Statuses in Simmental Cows and Describing Related Milk Fatty Acids and Relevant Associated Factors.

Anne M. Reus, Franziska E. Hajek, Simone M. Gruber, Stefan Plattner, Sabrina Hachenberg, Emil A. Walleser, Srikanth R. Aravamuthan, Rolf Mansfeld, Dörte Döpfer

Clinic for Ruminants with Ambulatory and Herd Health Services

Centre of Clinical Veterinary Medicine of the Ludwig-Maximilians-University Munich

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Differentiating between metabolic health statuses in Simmental cows and describing related milk fatty acids and relevant associated factors

Anne M. Reus,[*,1,] Franziska E. Hajek,[*,] Simone M. Gruber,[*,] Stefan Plattner,[*,] Sabrina Hachenberg,[†,] Emil A. Walleser,[‡,] Srikanth R. Aravamuthan,[‡,] Rolf Mansfeld,[*,2,] and Dörte Döpfer[‡,2,]

[*]Clinic for Ruminants with Ambulatory and Herd Health Services, LMU Munich, 85764 Oberschleissheim, Germany
[†]Deutscher Verband für Leistungs- und Qualitätsprüfungen e.V., 53113 Bonn, Germany
[‡]Department of Medical Science, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI 53706, USA

[1]Corresponding author: anne.reus@campus.lmu.de
[2]These authors contributed equally.

ABSTRACT

The aim of this observational study was to examine differences in milk fatty acid (**FA**) concentrations for different metabolic health statuses and for associated factors—specifically to examine with which FA concentrations an increased risk for developing a poor metabolic adaptation syndrome (**PMAS**) was associated. During weekly visits over 51 wk, blood samples were collected from cows between 5 and 50 days in milk. The farmer collected corresponding milk samples from all voluntary milkings. The analysis was performed on n = 2,432 samples from n = 553 Simmental cows. The observations were assigned to five different cow types (healthy, clever, athletic, hyperketonemic, and PMAS, representing five metabolic health statuses), based on the thresholds of 0.7 mmol/L, 1.2 mmol/L, and 1.4 for the concentrations of β-hydroxybutyrate and nonesterified fatty acids and for the milk fat-to-protein ratio, respectively. Linear regression models using the predictor variables cow type, parity, week of lactation, and milk yield as fixed effects were developed using a stepwise forward selection to test for significant associations of predictor variables regarding FA concentrations in milk. There was a significant interaction term found between PMAS cows and parity compared to healthy cows for C18:1 ($P < 0.001$) and for C18:0 ($P < 0.01$). It revealed higher concentrations for PMAS in primiparous and multiparous cows compared to healthy cows, the slope being steeper for primiparous cows. Further, an interaction term was found between PMAS cows and milk yield compared to healthy cows and milk yield for C16:0 ($P < 0.05$), revealing a steeper slope for the decrease of C16:0 concentrations with increasing milk yield for PMAS compared to healthy cows. The significant associations and interaction terms between cow type, parity, week of lactation, and milk yield as predictor variables and C16:0, C18:0, and C18:1 concentrations suggest excellent opportunities for cow herd health screening during the early postpartum period.

LAY SUMMARY

The focus of this observational study was to examine with which milk fatty acid (**FA**) concentrations an increased risk for developing a poor metabolic adaptation syndrome (**PMAS**) was associated. Poor metabolic adaptation syndrome is a condition to which high-yielding dairy cows are most susceptible during the first weeks after calving. Further, relevant associated factors (parity, week of lactation, and milk yield) were examined. The collected milk and corresponding blood samples were assigned to five different cow types (healthy, clever, athletic, hyperketonemic, and PMAS, representing five metabolic health statuses), based on the concentrations of β-hydroxybutyrate and nonesterified FA and on the milk fat-to-protein ratio. There was a significant interaction term found between PMAS cows and parity compared to healthy cows. The analyses revealed higher FA concentrations for PMAS primiparous and multiparous cows compared to healthy cows, with the slope being steeper for primiparous cows. Further, an interaction term was found between PMAS cows and milk yield compared to healthy cows and milk yield, revealing a steeper slope for the decrease of the FA C16:0 concentrations with increasing milk yield for PMAS compared to healthy cows. The significant associations and interaction terms between cow type, parity, week of lactation, and milk yield suggest excellent opportunities for cow herd health screening during the early postpartum period.

Key words: metabolic health status, milk fatty acids, poor metabolic adaptation syndrome

INTRODUCTION

Metabolic disorders, especially after calving, are a major factor when considering animal health and the economic aspects of modern dairy farming (Geishauser et al., 2001; Suthar et al., 2013; Gohary et al., 2016). Among other terms, the term "poor metabolic adaptation syndrome" (**PMAS**) was introduced to describe the complex metabolic status during a metabolic disorder, rather than ketosis, which, per its definition, is limited to an increase in ketone bodies, mostly β-hydroxybutyrate (**BHBA**), in the blood (Tremblay et al., 2018). Differences among high-, moderate-, and low-risk PMAS, mostly characterized by the nonesterified fatty acids (**NEFA**) concentration in blood, as well as different cow types,

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referring to different metabolic health statuses are described (Tremblay et al., 2018; Mandujano Reyes et al., 2023). The PMAS risk can be predicted using Fourier transform infrared spectroscopy (**FTIR**) data from milk (Tremblay et al., 2018).

Models predicting concentrations of NEFA and BHBA, as well as PMAS from concentrations of milk ingredients, have reached reasonable prediction accuracies (Tremblay et al., 2019); nevertheless, it is important to further understand the metabolic changes in the body after parturition to derive refined management instructions for cows with metabolic disorders and herd health management.

Fatty acids (**FA**) are promising because they reflect the extent of body fat mobilization (Rukkwamsuk et al., 2000; Oetzel, 2004). Concentrations of FA or FA ratios can be examined in blood and in milk (Mann et al., 2016; Melendez et al., 2016; Dorea et al., 2017; Reus and Mansfeld, 2020). Metabolized adipose tissue, which is present in the blood at higher concentrations and is used for milk production during a state of energy deficiency, consists of long-chain fatty acids, while FA directly synthesized in the mammary gland consist of short-chain fatty acids (Bauman and Griinari, 2003). Additionally, since milk is a substrate that is easy to obtain, it has a possible use in routine diagnoses using high-throughput technologies (Enjalbert et al., 2001; Mann et al., 2016).

The prevalence of metabolic disorders and the severity of the cases are influenced by environmental and management factors, such as feed and herd health monitoring, as well as by cow factors, such as the number of lactations, milk yield, days in milk (**DIM**), and the ability to tolerate hyperketonemia (Herdt, 2000; Gordon et al., 2013).

The aim of this observational study was to examine differences in the milk FA concentrations for different metabolic health statuses and for associated factors, such as parity, week of lactation, and milk yield in early postpartum cows—specifically to examine with which FA concentrations an increased risk for developing PMAS was associated.

MATERIALS AND METHODS

The official number of the approved animal experiment proposal for the Government of Bavaria was ROB-55.2Vet-2532. Vet_03-17-84. All used animal procedures apply to § 7-10 Tierschutzgesetz (TierSchG) and § 31-42 Tierschutzversuchstierverordnung (TierSchVersV).

Data Collection

Eight Bavarian dairy farms were selected to participate in the observational study. Selection criteria were location in the region of South Bavaria, use of an automated milking system (**AMS**) implying a minimum number of 50 lactating cows, herds consisting mainly of Simmental cows and willingness of the farmer to participate.

During weekly visits over 51 wk between January and December 2018 on the farms, blood samples were collected from all cows between 5 and 50 DIM from the coccygeal vein into a blood collection tube (BD-Serum-Gel-Vacutainer, SST 2 advanced, 8.5 mL, BD, Heidelberg, Germany).

Farm and cow identification, date, breed, DIM, and parity were recorded and assigned to the respective blood collection tube identifications.

One farm used DeLaval (De Laval GmbH, Glinde, Germany), two farms used Lely (Lely Industries N.V., Maassluis, The Netherlands), and five farms used Lemmer-Fullwood (Lemmer-Fullwood GmbH, Lohmar, Germany) AMS. The farmer connected a milk sample shuttle ORI-Collector (SAYCA Automatizacion, Alcalá de Henares, Spain) for 12 to 24 h, alternating over the day or the night before the visit, to collect composite milk samples from all voluntary milkings from cows between 5 and 50 DIM.

For the milk sample collection, sampling bottles of type 6845-xx (Bartec Benke GmbH, Gotteszell, Germany) containing 2 mL of preservative gel consisting of <4% sodium azide, <3% bronopol (2-bromo-2-nitropropane-1,3-diol), and <0.2% chloramphenicol were used. If the sample collection from a cow over one sample period resulted in multiple milk samples, the milk sample with the shortest time distance to the blood sampling was assigned to the blood sample.

Milk yield was measured by the standard equipment of the respective AMS, transmitted to the Dairy Herd Improvement Association of Bavaria (LKB Bayern e.V.) and assigned to the respective milk sample.

Blood samples were analyzed for concentrations of BHBA and NEFA in the laboratory of the Clinic for Ruminants, LMU Munich, using a Cobas c311-Analyzer (Roche Diagnostics, Rotkreuz, Switzerland).

Milk samples were transported at 4 °C and analyzed in the laboratory of the Bavarian Association for raw milk testing (MPR Bayern e. V., mpr, Wolnzach, Germany) for concentrations of fat, protein, urea, lactose, BHBA, and NEFA using the MilkoScan FT-6000 (FOSS GmbH, Hamburg, Germany). The somatic cell count (SCC) was determined using Fossomatic FC (FOSS GmbH), and the milk fat-to-protein ratio (**FPR**) was calculated.

The milk FA concentrations were calculated using milk FTIR data (Schwarz et al., 2021) by mpr using the FOSS-AN0064r7 package (FOSS GmbH).

Data Editing and Analysis

From n = 3,552 total observations, observations without standardization, blood BHBA or NEFA information, and FA panels with DIM < 5 or DIM > 50 and from other breeds were removed.

After removing observations without information on FA concentrations in milk, assuming the missing values to be random, n = 2,432 observations from n = 524 cows with a mean value of n = 4 observations (SD = 1.70) per cow were used for linear models for the outcomes of the milk FA concentrations. The analyses were performed using R software, versions 3.6.1 and 3.6.3 (R Development Core Team, 2013).

Cow Type Determination

Every observation was assigned to one of five different cow types (healthy, clever, athletic, hyperketonemic, and PMAS) following the decision tree in Figure 1 based on the description in Mandujano Reyes et al. (2023) (Figure 2). This implies that cows can be assigned to various cow types over time after parturition. Elevated cutoff values were set at 0.7 and 1.2 mmol/L for NEFA and BHBA, respectively, and the cutoff value for a reduced FPR was set at 1.4.

The healthy cow type was defined by normal BHBA and NEFA concentrations as well as a normal FPR. The clever cow type was defined by a normal BHBA concentration and a reduced FPR. Athletic cows were defined by an elevated BHBA concentration and a normal FPR, while hyperketonemic cows were defined by an elevated BHBA concentration and a reduced FPR. The PMAS cow type was defined by a normal

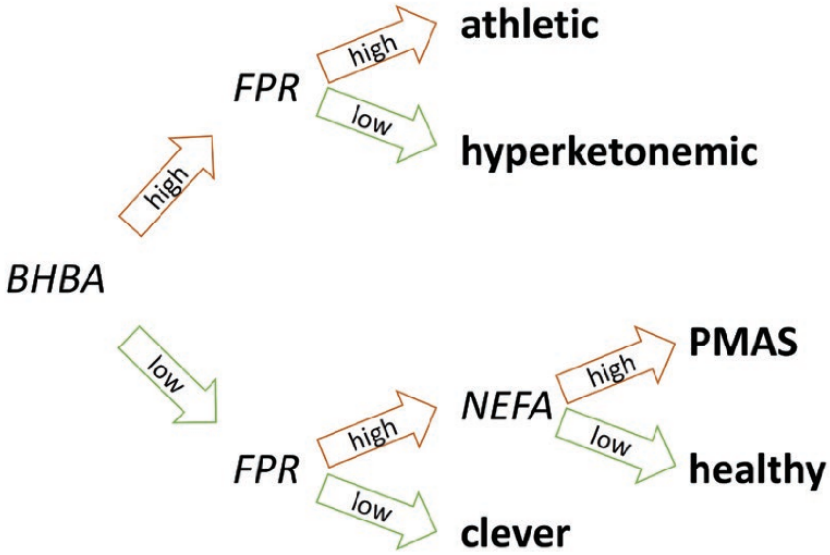

Figure 1. Decision tree to classify observations into the athletic, hyperketonemic, poor metabolic adaptation syndrome (PMAS), healthy, or clever cow types. Elevated cutoff values were set at 0.7 and 1.2 mmol/L for nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA), respectively. The cutoff value for an elevated milk fat-to-protein ratio (FPR) was set at 1.4.

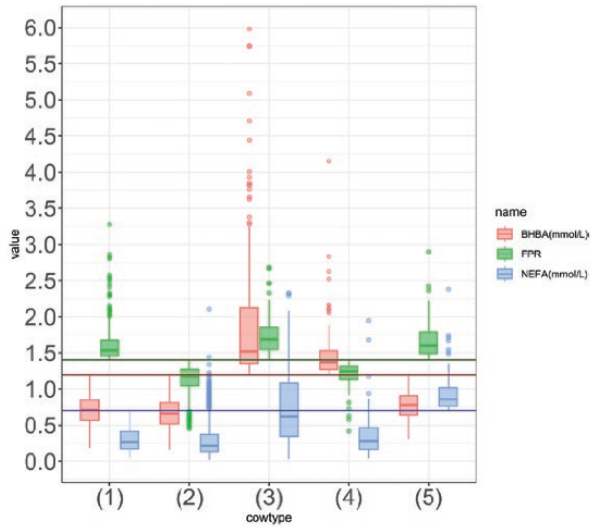

Figure 2. Boxplot of the nonesterified fatty acid (NEFA) and β-hydroxybutyrate (BHBA) concentrations in mmol/L as well as the fat-to-protein ratio (FPR) of the five cow types in the data set (n = 2,432 observations from 521 Simmental cows from eight Bavarian herds sampled at 5 to 50 days in milk). Cow types of observations are classified following the decision tree shown in Figure 1 resulting in the following group sizes: healthy: n = 539, clever: n = 1,584, athletic: n = 146, hyperketonemic: n = 82, poor metabolic adaptation syndrome (PMAS): n = 81. Cowtypes: (1): healthy, (2): clever, (3): athletic, (4): hyperketonemic, and (5): poor metabolic adaptation syndrome (PMAS).

BHBA concentration and a normal FPR and, in contrast to the healthy cow type, by an elevated NEFA concentration.

Linear Models

Days in milk were transformed into a categorial variable: week of lactation. Weeks 1, 2, 3, 4, 5, 6, 7, and 8 of lactation were defined as DIM ≤ 7, 8–14, 15–21, 22–28, 29–35, 36–42, 43–49, and 50, respectively.

Table 1. Descriptive statistics of cow production variables from the data set of eight dairy herds in Bavaria sampled between 5 and 50 days in milk. Data set (n = 2,432 observations)

Variable	Mean	SD
Lactation number	3.2	(1.9)
Days in milk	28.3	(12.8)
Milk yield per day, kg	33.2	(8.0)
Fat, %	4.2	(0.9)
Protein, %	3.3	(0.4)
Lactose, %	4.9	(0.2)
Urea, mg/100 mL	24.4	(7.3)
SCC, 1,000 cells/mL	187.2	(618.4)
BHBA, mmol/L	0.80	(0.48)
NEFA, mmol/L	0.34	(0.29)
FPR	1.30	(0.30)

SCC, somatic cell count in milk; BHBA, β-hydroxybutyrate in blood; NEFA, nonesterified fatty acids in blood; FPR, fat-to-protein ratio in milk.

Four predictor variables were defined: cow type (healthy, clever, athletic, hyperketonemic, and PMAS), parity (primiparous or multiparous cow), and week of lactation (week 1 to 8) as categorial, as well as milk yield in kg (milk yield) as a continuous variable. Milk yield was log transformed and milk yield and FA concentrations were scaled. Collinearity was checked between the predictor variables cow type, parity, DIM, and milk yield using an identity matrix created using the ggpairs function.

Three milk FA concentrations (palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1)) were defined as outcome variables. Histograms were used to assess whether FA concentrations were normally distributed within the cow types. The one-way Kruskal–Wallis test was used to test for differences in concentrations of FA in milk between cow types, and pairwise comparisons using Wilcoxon rank sum

Table 2. Mean milk fatty acid concentrations and standard deviation per cow type (n = 2,432 observations) in g/100 g milk for palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1) as calculated using milk FTIR and the standard error (SE) of the prediction

	Healthy (n = 539)	Clever (n = 1,584)	Athletic (n = 146)	Hyperketonemic (n = 82)	PMAS (n = 81)	Total (n = 2,432)	SE (prediction)
C16:0	1.30^a (0.22)	1.07^b (0.19)	1.22^c (0.20)	1.01^b (0.20)	1.23^c (0.22)	1.13 (0.22)	0.11
C18:0	0.59^a (0.11)	0.41^b (0.10)	0.70^c (0.15)	0.45^d (0.11)	0.68^c (0.14)	0.48 (0.15)	0.05
C18:1	1.40^a (0.33)	0.90^b (0.25)	1.73^c (0.44)	0.98^b (0.31)	1.74^c (0.42)	1.09 (0.41)	0.07

PMAS, poor metabolic adaptation syndrome;16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid.
$^{a-d}$Letters indicate mean fatty acid concentrations differ as determined by pairwise comparisons using Wilcoxon rank sum test with a Bonferroni correction ($P < 0.05$).

Table 3. Summary of the linear regression for palmitic acid (C16:0) in milk in g/100 g fat without random effects, observations: n = 2,432

C16:0	Mean (SD)	Coefficient (95% CI, P-value)
Cowtype		
Healthy (= ref)	0.8 (1.0)	—
Clever	−0.3 (0.8)	−1.04 (−1.13 to −0.95, $P < 0.001$)
Athletic	0.4 (0.9)	−0.36 (−0.53 to −0.20, $P < 0.001$)
Hypket.	−0.6 (0.9)	−1.31 (−1.52 to −1.11, $P < 0.001$)
PMAS	0.5 (1.0)	−0.30 (−0.51 to −0.09, $P < 0.005$)
Parity		
Multip. (= ref)	−0.0 (1.0)	—
Healthy × Multip. (= ref)		—
Clever × Multip.		−1.06 (−1.14 to −0.97, $P < 0.001$)
Athletic × Multip.		−0.34 (−0.51 to −0.17, $P < 0.001$)
Hypket. × Multip.		−1.32 (−1.51 to −1.13, $P < 0.001$)
PMAS × Multip.		−0.19 (−0.39 to 0.01, $P < 0.057$)
Primip.	0.0 (1.0)	0.02 (−0.08 to 0.11, $P < 0.722$)
Healthy × Primip. (= ref)		—
Clever × Primip.		−1.03 (−1.18 to −0.87, $P < 0.001$)
Athletic × Primip.		0.05 (−0.27 to 0.37, $P < 0.740$)
Hypket. × Primip.		−0.98 (−1.67 to −0.30, $P < 0.005$)
PMAS × Primip.		0.15 (−0.52 to 0.22, $P < 0.433$)
log(milk yield) [−11.3, 3.0]	0.0 (1.0)	−0.21 (−0.25 to −0.18, $P < 0.001$)
Healthy × log(milk yield) (= ref)		—
Clever × log(milk yield)		−0.22 (−0.27 to −0.17, $P < 0.001$)
Athletic × log(milk yield)		−0.12 (−0.26 to 0.01, $P < 0.065$)
Hypket. × log(milk yield)		−0.11 (−0.35 to 0.12, $P < 0.346$)
PMAS × log(milk yield)		−0.21 (−0.40 to −0.03, $P < 0.026$)

ref, reference; hypket., hyperketonemic; PMAS, poor metabolic adaptation syndrome; primip., primiparous; multip., multiparous.

test with a Bonferroni correction were conducted to test for differences between each cow type (Table 1).

The models were built using the package lme4. Linear regression models using the four predictor variables and interaction terms between cow type and the other predictor variables as fixed effects, farm identification (ID) and cow ID as random effects and the FA concentrations as outcome variable were developed with the data set using forward stepwise selection. This resulted in three models for C16:0, C18:0, and C18:1 in milk, respectively.

A goodness-of-fit evaluation was performed by plotting residual over fitted values for each model. The resulting graph showed that the residual values were distributed symmetrically over the horizontal line at 0. The distribution of the residuals was normal.

Visualization

Effect plots were created using the allEffects function of the effects package to visualize the association of the predictor variables with the cow types regarding FA concentrations.

Table 4. Summary of the linear regression for stearic acid (C18:0) in milk in g/100 g fat without random effects, observations: n = 2,432

C18:0	Mean (SD)	Coefficient (95% CI, P-value)
Cowtype		
Healthy (= ref)	0.8 (0.8)	—
Clever	−0.5 (0.7)	−1.25 (−1.32 to −1.17, P < 0.001)
Athletic	1.5 (1.0)	0.70 (0.57 to 0.83, P < 0.001)
Hypket.	−0.2 (0.7)	−0.94 (−1.11 to −0.77, P < 0.001)
PMAS	1.4 (0.9)	0.58 (0.41 to 0.76, P < 0.001)
Parity		
Multip. (= ref)	−0.1 (1.0)	—
Healthy × Multip. (= ref)		—
Clever × Multip.		−1.01 (−1.17 to −1.03, P < 0.001)
Athletic × Multip.		0.61 (0.47 to.75, P < 0.001)
Hypket. × Multip.		−0.86 (−1.02 to −0.70, P < 0.001)
PMAS × Multip.		0.23 (0.06 to 0.39, P < 0.007)
Primip.	0.3 (1.0)	0.43 (0.34 to 0.53, P < 0.001)
Healthy × Primip. (= ref)		—
Clever × Primip.		−1.14 (−1.27 to −1.02, P < 0.001)
Athletic × Primip.		0.13 (−0.13 to 0.38, P < 0.341)
hypket. × Primip.		−0.74 (−1.30 to −0.19, P < 0.009)
PMAS × Primip.		0.40 (0.10 to 0.70, P < 0.009)
Week		
1 (= ref)	0.4 (1.1)	—
2	0.6 (1.0)	0.18 (−0.01 to 0.38, P < 0.068)
3	0.3 (1.0)	−0.15 (−0.34 to 0.04, P < 0.120)
4	0.0 (0.9)	−0.41 (−0.60 to −0.22, P < 0.001)
5	−0.1 (1.0)	−0.53 (−0.72 to −0.34, P < 0.001)
6	−0.3 (0.9)	−0.74 (−0.93 to −0.55, P < 0.001)
7	−0.4 (0.8)	−0.80 (−0.99 to −0.61, P < 0.001)
8	−0.5 (0.8)	−0.92 (−1.25 to −0.59, P < 0.001)
log(milk yield) [−11.3,3.0]	−0.0 (1.0)	−0.17 (−0.21 to −0.13, P < 0.001)
Healthy × log(milk yield) (= ref)		—
Clever × log(milk yield)		0.01 (−0.03 to 0.05, P < 0.590)
Athletic × log(milk yield)		−0.19 (−0.29 to −0.08, P < 0.001)
Hypket. × log(milk yield)		−0.04 (−0.23 to 0.15, P < 0.678)
PMAS × log(milk yield)		−0.07 (−0.22 to 0.08, P < 0.363)
Multip. × log(milk yield) (= ref)		—
Primip. × log(milk yield)		−0.07 (−0.16 to 0.02, P < 0.151)

ref, reference; hypket., hyperketonemic; PMAS, poor metabolic adaptation syndrome; primip., primiparous; multip., multiparous.

RESULTS

Descriptive Statistics

Descriptive statistics have been summarized in Table 1. Of n = 2,432 total observations, n = 539, n = 1,584, n = 146, n = 83, and n = 81 were assigned to the healthy, clever, athletic, hyperketonemic, and PMAS cow types, respectively (Table 2).

The C16:0, C18:0, and C18:1 concentrations varied significantly (P < 0.05) between cow types as determined by Kruskal–Wallis test. Significant differences in concentrations between each cow type determined by pairwise comparisons using Wilcoxon rank sum test with a Bonferroni correction are indicated in Table 2. The standard error (SE) for each parameter prediction is also indicated.

Linear Models

The final models varied in characteristics (Tables 3–5). All three final models used parity as a fixed effect and interaction terms between cow type on the one hand, and parity and log(milk yield) on the other hand. Models for C18:0 and C18:1 also used week as fixed effect and the model for C18:0 additionally used the interaction term between parity and log(milk yield). The results for the respective effects are described below.

Associations for Cow Type

All associations for cow type were significant (P < 0.01). Associations for C16:0 revealed lower concentrations for PMAS cows compared to healthy cows (P < 0.01) and

Table 5. Summary of the linear regression for oleic acid (C18:1) in milk in g/100 g fat with random effects (cow ID, farm ID), observations: n = 2,432

C18:1	Mean (SD)	Coefficient (95% CI, P-value)
Cowtype		
Healthy (= ref)	0.8 (0.8)	—
Clever	−0.5 (0.6)	−1.23 (−1.30 to −1.16, P < 0.001)
Athletic	1.5 (1.1)	0.78 (0.65 to 0.91, P < 0.001)
Hypket.	−0.3 (0.7)	−1.03 (−1.20 to −0.87, P < 0.001)
PMAS	1.6 (1.0)	0.80 (0.64 to 0.97, P < 0.001)
Parity		
Multip. (= ref)	−0.1 (1.0)	—
Healthy × Multip. (= ref)		—
Clever × Multip.		−1.13 (−1.19 to −1.07, P < 0.001)
Athletic × Multip.		0.71 (0.59 to 0.83, P < 0.001)
hypket. × Multip.		−0.92 (−1.06 to −0.78, P < 0.001)
PMAS × Multip.		0.37 (0.23 to 0.51, P < 0.001)
Primip.	0.3 (1.1)	0.44 (0.35 to 0.54, P < 0.001)
Healthy × Primip. (= ref)		—
Clever × Primip.		−1.19 (−1.30 to −1.08, P < 0.001)
Athletic × Primip.		0.16 (−0.07 to 0.38, P < 0.248)
Hypket. × Primip.		−0.72 (−1.21 to −0.24, P < 0.014)
PMAS × Primip.		0.69 (0.43 to 0.95, P < 0.001)
Week		
1 (= ref)	0.3 (1.2)	—
2	0.5 (1.1)	0.20 (0.00 to 0.40, P < 0.047)
3	0.2 (1.0)	−0.08 (−0.27 to 0.12, P < 0.441)
4	−0.0 (0.9)	−0.33 (−0.52 to −0.13, P < 0.001)
5	−0.1 (1.0)	−0.40 (−0.59 to −0.21, P < 0.001)
6	−0.3 (0.9)	−0.58 (−0.77 to −0.39, P < 0.001)
7	−0.3 (0.8)	−0.61 (−0.81 to −0.42, P < 0.001)
8	−0.4 (0.8)	−0.76 (−1.09 to −0.42, P < 0.001)
log(milk yield) [−11.3,3.0]	0.0 (1.0)	−0.17 (−0.21 to −0.13, P < 0.001)
Healthy × log(milk yield) (= ref)		
Clever × log(milk yield)		−0.01 (−0.04 to 0.02, P < 0.684)
Athletic × log(milk yield)		−0.29 (−0.38 to −0.20, P < 0.001)
Hypket. × log(milk yield)		−0.04 (−0.21 to 0.13, P < 0.707)
PMAS × log(milk yield)		−0.13 (−0.26 to 0.01, P < 0.120)

Ref, reference; hypket., hyperketonemic; PMAS, poor metabolic adaptation syndrome; primip., primiparous; multip., multiparous.

descending in the following order: athletic, clever, and hyperketonemic compared to healthy cows (P < 0.001, Table 3, Figure 3). For C18:0, associations revealed lower concentrations for hyperketonemic and even lower for clever compared to healthy cows (P < 0.001) and higher for PMAS and even higher for athletic compared to healthy cows (P < 0.001, Table 4, Figure 4). Finally, associations for C18:1 revealed similar association to C18:0, only PMAS cow type concentrations were higher than athletic cow type concentrations and all associations were significant (P < 0.001, Table 5, Figure 5).

Associations for Parity

All FA showed associations for parity revealing higher concentrations in primiparous compared to multiparous cows. These associations were significant only for C18:0 and C18:1 (P < 0.001, Tables 3–5, Figures 3–5).

Significant interaction terms between parity and cow types were found for all FA. The slopes between cow types were similar between the associations for cow type only and the interaction term between parity and cow type for multiparous cows for C16:0 and C18:0 and for primiparous cows for C18:1. Apart from PMAS cows within C16:0 and athletic cows within C18:1, this was statistically significant for all mentioned slopes (P < 0.01). For C16:0, the slopes for primiparous cows were different for athletic cows revealing higher concentrations in athletic compared to healthy cows and a less steep slope for hyperketonemic than for clever cows, both showing lower concentrations, compared to healthy cows. In primiparous cows, only the associations between clever and hyperketonemic on the one hand compared to healthy on the other hand are statistically significant (P < 0.01). For C18:0, the slopes for primiparous compared to multiparous cows were different for the interaction term with athletic and PMAS cows, PMAS cows revealing a steeper slope and athletic cows revealing a less steep slope, both showing higher concentrations than healthy cows. The association between parity and cow type was statistically significant for clever (P < 0.001) and hyperketonemic cows and PMAS cows (P < 0.01). The slopes within C18:1 in multiparous compared to primiparous cows were different for athletic and PMAS cows, revealing a less steep slope for PMAS than for athletic cows, both still showing higher concentrations compared to healthy cows. All interaction terms between multiparous cows and cow type were statistically significant for C18:1 (P < 0.001).

Associations for Week of Lactation

There were significant associations between week of lactation and C18:0 and C18:1 concentrations (P < 0.05, Tables 4 and 5, Figures 4 and 5), revealing a peak during week 2, that was only significant for C18:1 (P < 0.05) and a following decrease over increasing weeks of lactation that was significant for C18:0 beginning in week 3 (P < 0.001) and for C18:1 beginning in week 4 (P < 0.001).

Associations for Milk Yield

All FA concentrations decreased with increasing milk yield (P < 0.001, Tables 3–5, Figures 3–5).

An interaction term between milk yield and cow type was used for all FA and revealed increasingly steep slopes in the following order for C16:0: hyperketonemic, athletic, PMAS, and clever compared to healthy cows. These findings were significant for PMAS (P < 0.05) and clever cows (P < 0.01). For C18:0 and C18:1, the order was as following: clever, hyperketonemic, PMAS, and athletic compared to healthy cows and statistically significant only for athletic cows (P < 0.005).

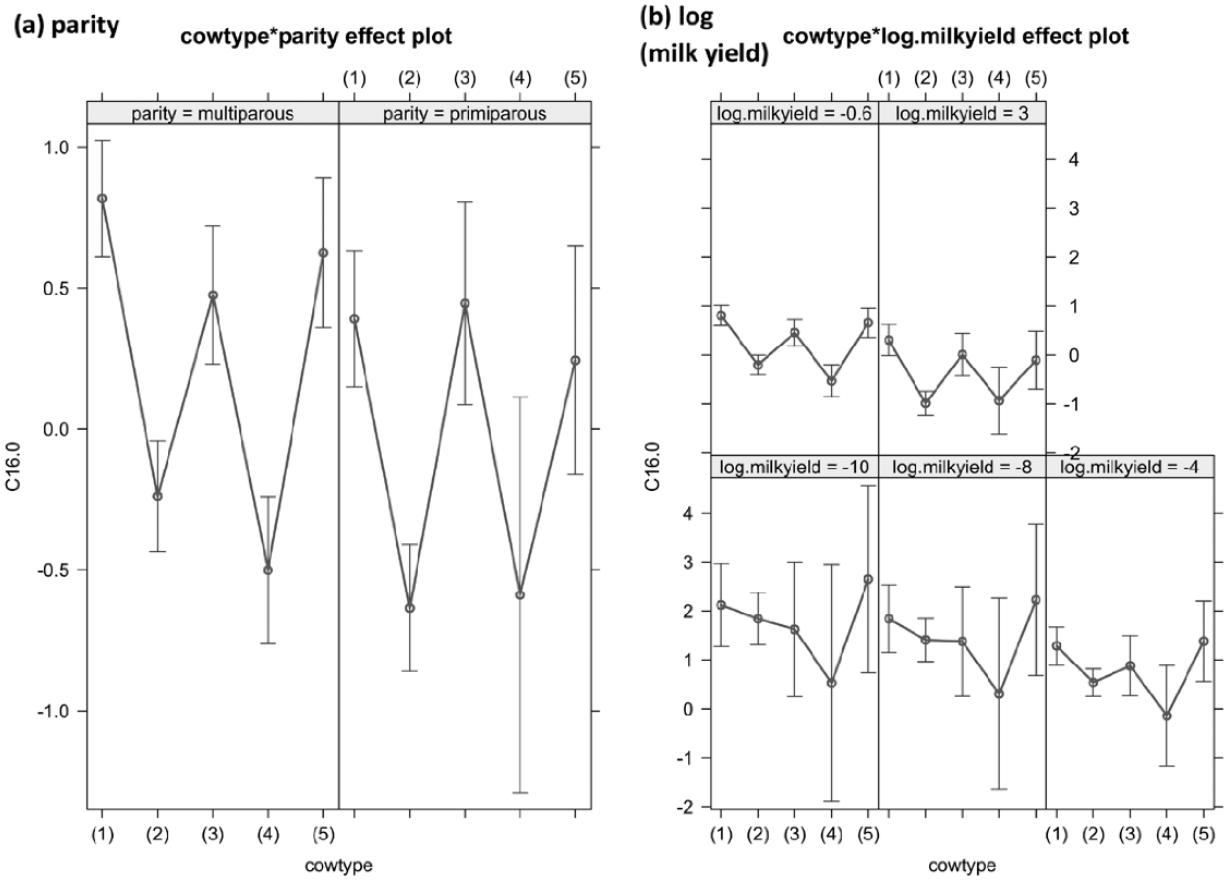

Figure 3. Effect plots of the linear model for scaled palmitic acid (C16:0) concentration in milk (n = 2,432 observations, originally in g/100 g fat) with 95% confidence intervals. Effects: (a) parity and (b) log(milk yield), originally in L/d. The cow types are as following: (1): healthy, (2): clever, (3): athletic, (4): hyperketonemic, and (5): poor metabolic adaptation syndrome (PMAS).

There were no significant findings for the interaction term between parity and milk yield within C18:0.

DISCUSSION

Examined Fatty Acids and Effects

Milk short-chained fatty acids (C5–C15) decrease with increasing blood NEFA or ketone bodies as a biomarker for metabolic disorders, while long-chained fatty acids, for example, oleic acid (C18:1), increase during metabolic disorders in blood (Bauman and Griinari, 2003; Van Haelst et al., 2008; Mann et al., 2016). The present results, for example, higher concentrations of C18:0 and C18:1 in the milk of PMAS cows, are in accordance with the finding that long chained fatty acids increase with an increased risk of a metabolic imbalance. It was found that stearic acid (C18:0) is the predominant fatty acid in the uptake through feed (Glasser et al., 2008; Loften et al., 2014), while oleic acid is the main fatty acid in adipose tissue and the first FA released during NEB (Rukkwamsuk et al., 2000; Loften et al., 2014). Palmitic acid (C16:0) was shown to be the predominant fatty acid in milk fat, with C18:1 representing the second highest concentration (Loften et al., 2014). This is in accordance with the results of this study for the total set of data and for the clever and hyperketonemic cow group. For healthy, athletic, and PMAS cows, the concentration of oleic acid was higher than the concentration of palmitic acid.

When comparing blood and especially milk FA concentrations of primiparous to multiparous cows during early lactation, several reports can be found in the respective literature. It has been reported that C18:0 and C18:1 would be higher in primiparous than in multiparous cows (Van et al., 2020). In accordance with these findings, our results indicate that C18:0 and C18:1 milk fat concentration were significantly ($P < 0.001$) higher in primiparous than in multiparous cows. Multiparous cows suffer more often from metabolic diseases than primiparous cows (Gordon et al., 2013).

The associations for C16:0 concentrations ($P < 0.001$) revealed very similar concentrations for multiparous compared to primiparous cows when not considering different cow types. The findings for C16:0 and C18:0 are in accordance with Van et al. (2020).

It is well known that cows suffer from NEB after parturition (Bell, 1995). Furthermore, milk FA and FA group concentrations physiologically decrease over increasing weeks of lactation (Gross et al., 2011). These facts agree with our results, as C18:0 and C18:1 concentration decreased over time after parturition.

Interaction terms between cow type and milk yield revealed decreased concentrations of milk FA in association with increased milk yield for every cow type. This contradicts previous findings, showing that high-yielding cows were exposed to higher metabolic stress (Nogalski et al., 2015). The interaction terms revealed significantly steeper slopes of decrease

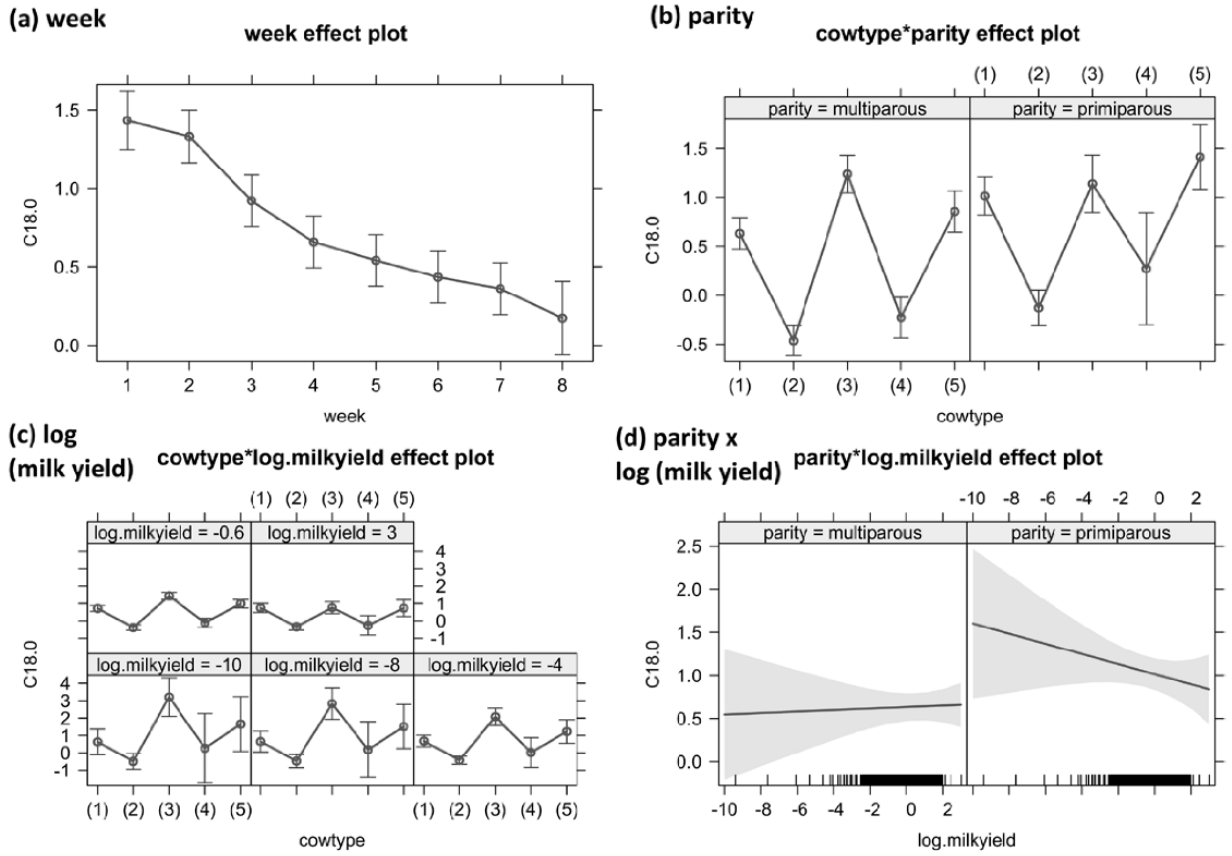

Figure 4. Effect plots of the linear model for scaled stearic acid (C18:0) concentration in milk (n = 2,432 observations, originally in g/100 g fat) with 95% confidence intervals. Effects: (a) week after parturition, (b) parity, (c) log(milk yield), originally in L/d, and (d) parity × log(milk yield), originally in L/d. The cow types are as following: (1): healthy, (2): clever, (3): athletic, (4): hyperketonemic, and (5): poor metabolic adaptation syndrome (PMAS).

for PMAS compared to healthy cows for C16:0 ($P < 0.05$) and steeper slopes of decrease for PMAS compared to healthy cows for C18:0 and C18:1, although these findings were not statistically significant, leading to a greater difference between PMAS compared to healthy cows associated with lower milk yield and more similar concentrations associated with higher milk yield. The findings support the reports in the literature that, during a high milk yield, all cow types are exposed to metabolic imbalances (Nogalski et al., 2015), and show that PMAS cows are more severely affected than healthy cows when associated with lower milk yield compared to higher milk yield. Consequently, these findings support the idea of analyzing the response patterns to NEB by cow type, or these different patterns could go unnoticed and improve the differentiation between PMAS and non-PMAS cows when associated with lower milk yield.

Application

There were significant associations and interaction terms between the predictor variables cow type, parity, week of lactation and milk yield and the outcome variables C16:0, C18:0, and C18:1 concentration.

These findings represent excellent opportunities for cow herd health screening during the early postpartum period. Transition cow management aims to prevent the consequences of NEB to improve productivity, cow health, and longevity. Preventing the consequences of NEB in PMAS cows by applying early detection followed by intervention could prevent animal suffering. Therefore, cow typing contributes to cow health and welfare while guarding the economic interests of producers, particularly when informed by the predictor variables parity, week of lactation, and milk yield.

Contributing to the opportunity to use cow types for early lactation herd health screening is the availability of high-throughput FTIR technologies, which minimize the costs and time needed for milk sample analysis.

Limitations

Various technical challenges, such as the high number of people involved in taking, shipping, and analyzing the samples or non-analyzable samples, led to observations that missed relevant information, such as blood BHBA or NEFA concentrations or milk FA panels. These observations had to be removed from the data analyses. Since the missing values were assumed to be at random and each observation was considered as standing on its own, no effects of the missing values on the results were to be expected.

CONCLUSIONS

There was a significant interaction term found between PMAS cows and parity compared to healthy cows for C18:1 ($P < 0.001$) and for C18:0 ($P < 0.01$). It revealed higher concentrations for PMAS in primiparous and multiparous

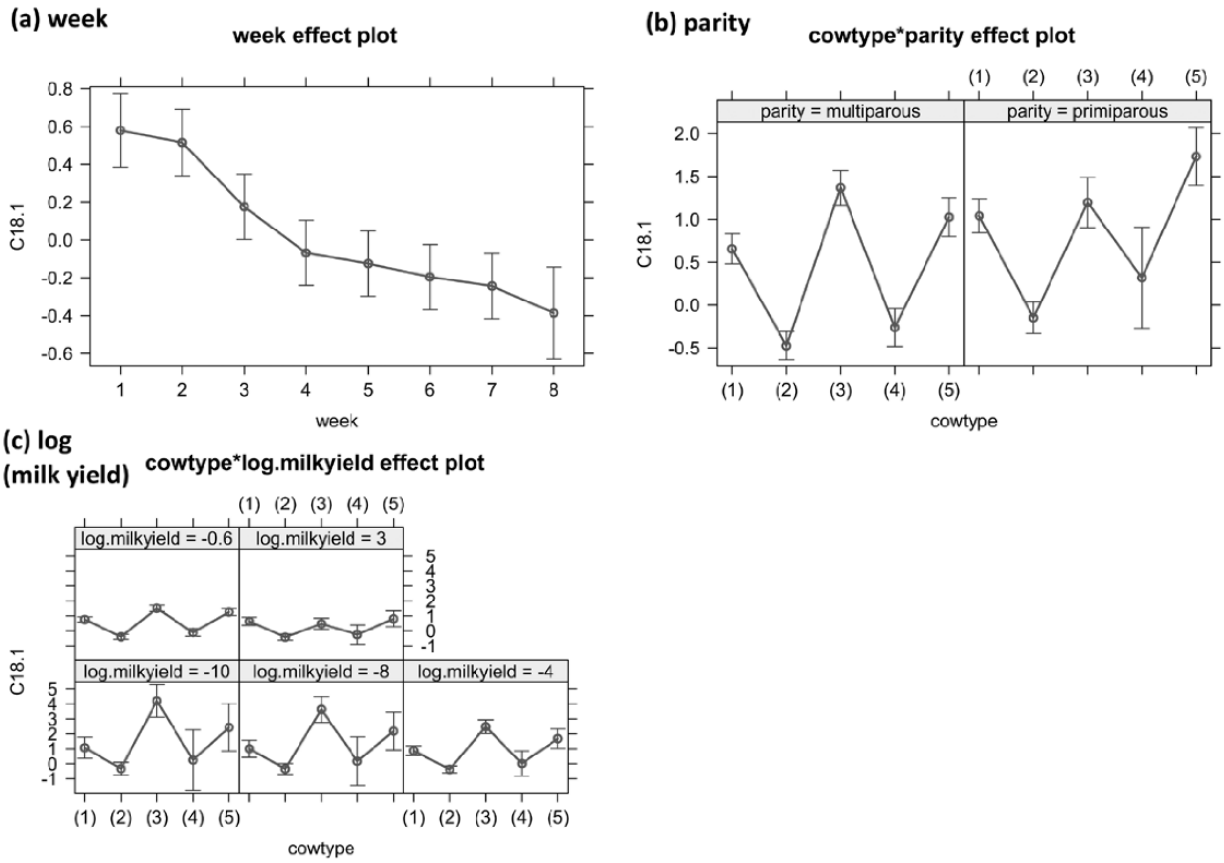

Figure 5. Effect plots of the linear model for scaled oleic acid (C18:1) concentration in milk (n = 2,432 observations, originally in g/100 g fat) with 95% confidence intervals. Effects: (a) week of lactation (week), (b) parity, and (c) log(milk yield), originally in L/d. The cow types are as following: (1): healthy, (2): clever, (3): athletic, (4): hyperketonemic, and (5): poor metabolic adaptation syndrome (PMAS).

cows compared to healthy cows, the slope being steeper for primiparous cows. Further, an interaction term was found between PMAS cows and milk yield compared to healthy cows and milk yield for C16:0 ($P < 0.05$), revealing a steeper slope for the decrease of C16:0 concentrations with increasing milk yield for PMAS compared to healthy cows. The significant associations and interaction terms between cow type, parity, week of lactation, and milk yield as predictor variables and C16:0, C18:0, and C18:1 concentration suggest excellent opportunities for cow herd health screening during the early postpartum period.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA SHARING

Given that the data belong to the producers, it is not possible to share the data set widely. The data and software code may be shared upon request.

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IV. FURTHER RESULTS

In addition to the examinations conducted on milk FA concentrations described in the second publication (Reus et al., 2023), blood FA concentrations were examined and are described in the following. Materials and methods were the same as described in the second publication (Reus et al., 2023), concerning data collection, data editing and analysis, cow type determination and visualization. Blood FA concentrations were calculated using milk FTIR data and applying a regression model developed and validated by Qlip N.V. (Qlip N.V., Leusden, The Netherlands). Concerning the linear models, they were built according to the description in the second publication (Reus et al., 2023) but used blood FA concentrations (palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1)) instead of milk FA concentrations as outcome variables.

1. Descriptive Statistics

Descriptive statistics have been summarized in the second publication (Reus et al., 2023).

As for the milk FA, there were significant differences ($P < 0.05$) in blood C16:0, C18:0 and C18:1 concentrations between cow types as determined by Kruskal-Wallis test. Significant differences in concentrations between each cow type are indicated in Table 2. They were determined by pairwise comparisons using Wilcoxon rank sum test with a Bonferroni correction. The root mean squared error (RMSE) for each parameter prediction is also indicated.

Table 2: Mean blood fatty acid concentrations and standard deviation per cow type (n = 2432 observations) in µmol/L blood for palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) as calculated using milk FTIR data and applying a regression model developed and validated by Qlip N.V. (Qlip N.V., Leusden, The Netherlands) and the root mean squared error (RMSE) of the prediction.

	healthy (n = 539)	clever (n = 1584)	athletic (n = 146)	hyperketonemic (n = 82)	PMAS (n = 81)	total (n = 2432)	RMSE (prediction)
C16:0	72.31[a] (43.03)	31.02[b] (38.72)	130.30[c] (53.25)	63.85[a] (41.03)	121.76[c] (48.96)	50.26 (50.99)	18.9
C18:0	96.32[a] (39.27)	61.34[b] (38.09)	149.65[c] (45.86)	96.14[a] (35.77)	139.02[c] (42.95)	78.15 (47.05)	19.3
C18:1	120.49[a] (86.03)	29.73[b] (73.34)	226.57[c] (108.23)	81.32[d] (82.31)	216.24[c] (99.55)	69.61 (101.01)	38.2

PMAS: poor metabolic adaptation syndrome, C16:0: palmitic acid, C18:0: stearic acid, C18:1: oleic acid

[a–d] Letters indicate mean fatty acid concentrations differ as determined by pairwise comparisons using Wilcoxon rank sum test with a Bonferroni correction ($P < 0.05$)

2. Linear Models

Again, the final models for the blood FA varied in the fixed effects and interaction terms used (Tables 3-5). Alle three final models used parity and week as a fixed effect and interaction terms between cow type and parity as well as between cow type and log(milk yield). Models for C16:0 and C18:0 also used the interaction term between week and log(milk yield). The results for the respective effects and interaction terms are described below.

2.1. Associations for Cow type

As cow type was only used within interaction terms and not a fixed effect in all three models, the differences in mean values are described in this section.

All blood FA revealed similar differences in concentrations between cow types (Tables 3-5, Figures 1-3). Compared to healthy cows, hyperketonemic cows showed lower concentrations. This was significant only for C18:1 ($P < 0.001$). The clever cow type revealed even lower concentrations than hyperketonemic cows compared to the healthy cow type. This finding was significant for all FA ($P < 0.001$). Compared to healthy cows, PMAS and clever cows had

significantly ($P < 0.001$) higher FA concentrations, with clever cows showing the highest FA concentrations.

2.2. Associations for Parity

All blood FA revealed the same associations within this effect and the interaction term with cow type. Primiparous cows showed significantly ($P < 0.001$) higher C16:0, C18:0 and C18:1 concentrations compared to multiparous cows.

Within multiparous cows, the FA concentrations of each cow type had the same ratio as for the associations for cow type only. This finding was statistically significant ($P < 0.001$) for all cow types and all FA, except for hyperketonemic cows for C16:0, showing a lower p-value ($P < 0.01$), on one hand, and for C18:0, not being statistically significant, on the other hand.

Within primiparous cows, hyperketonemic and clever cows revealed the same ratio compared to healthy cows as for the association for cow type only and as within multiparous cows. This finding was statistically significant for clever cows only ($P < 0.001$). On the contrary, athletic cows revealed higher FA concentrations compared to healthy cows, not being statistically significant. Further, PMAS cows showed even higher concentrations than athletic compared to healthy cows ($P < 0.001$), thus changing the ratio of athletic and PMAS cows within primiparous cows compared to multiparous cows.

2.3. Associations for Week of Lactation

Significant associations were found for all blood FA. All three FA showed a peak in concentration in week 2, the difference compared to week 1 only being significant for C18:0 ($P < 0.05$). All FA concentrations decreased over increasing weeks of lactation. This was significant for C16:0 concentrations from week 3 on and for C18:0 concentrations from week 4 on ($P < 0.001$). C18:0 concentrations showed a significance in week 3 ($P < 0.05$) and from week 4 on ($P < 0.001$).

2.4. Associations for Milk Yield

All blood FA concentrations increased with increasing milk yield when considering mean values. This was statistically significant for C16:0 ($P < 0.01$) and for C18:0 concentrations ($P < 0.001$).

When considering the models, interaction terms were used for all blood FA. The slope was decreasing with increasing milk yield for athletic cows for C16:0 and C18:1 concentrations. This finding was, compared to the increasing slope of healthy cows, statistically significant only for C18:1 concentrations ($P < 0.001$). All other cow types showed steeper slopes compared to healthy cows in increasing concentrations in the following order: athletic (only C18:0), hyperketonemic, PMAS and clever cows. This was statistically significant for all FA for clever cows ($P < 0.001$) and for PMAS cows for C16:0 ($P < 0.05$) and for C18:0 concentrations ($P < 0.01$).

2.5. Interaction term between Week of Lactation and Milk Yield

Models for blood C16:0 and C18:0 concentrations used an interaction term between week of lactation and milk yield. This interaction term revealed less steep slopes of the increase of FA concentrations with increasing milk yield and even increasingly steep slopes of the decrease of FA concentrations over time after parturition compared to week 1, where the increase of FA concentrations with increasing milk yield was the steepest. An exception was week 5, where the second steepest decrease in FA concentrations was found for both C16:0 and C18:0 concentrations. These findings were significant for week 4 for C16:0 and week 2 for C18:0 concentrations ($P < 0.05$), from week 5 on for C16:0 and for weeks 3 and 8 for C18:0 concentrations ($P < 0.01$) and for weeks 4 – 7 for C18:0 concentrations ($P < 0.001$).

Table 3: Summary of the linear regression for scaled palmitic acid (C16:0) concentration in blood (originally in µmol/L blood) with random effects (cow ID, farm ID), observations: n = 2432.

C16:0	mean (SD)	difference in mean values (95% CI, P -value)	coefficient – model (95% CI, P -value)
cowtype			
healthy (= ref)	0.4 (0.8)	-	
clever	-0.4 (0.8)	-0.81 (-0.89 to -0.73, P < 0.001)	
athletic	1.6 (1.0)	1.14 (0.99 to 1.28, P < 0.001)	
hypket.	0.3 (0.8)	-0.17 (-0.35 to 0.02, P < 0.083)	
PMAS	1.4 (1.0)	0.97 (0.78 to 1.16, P < 0.001)	
parity			
multip. (= ref)	-0.1 (1.0)	-	
healthy x multip. (= ref)			-
clever x multip.			-0.74 (-0.82 to -0.67, P < 0.001)
athletic x multip.			0.94 (0.78 to 1.09, P < 0.001)
hypket. x multip.			-0.24 (-0.41 to -0.06, P < 0.008)
PMAS x multip.			0.51 (0.33 to 0.69, P < 0.001)
primip.	0.2 (0.9)	0.25 (0.15 to 0.35, P < 0.001)	0.35 (0.18 to 0.51, P < 0.001)
healthy x primip. (= ref)			-
clever x primip.			-0.63 (-0.77 to -0.49, P < 0.001)
athletic x primip.			0.18 (-0.11 to 0.47, P < 0.220)
hypket. x primip.			-0.32 (-0.94 to 0.29, P < 0.306)
PMAS x primip.			0.67 (0.34 to 1.00, P < 0.001)
week			
1 (= ref)	0.1 (1.3)	-	-
2	0.5 (1.1)	0.42 (0.23 to 0.62, P < 0.001)	0.04 (-0.10 to 0.18, P < 0.587)
3	0.3 (1.0)	0.22 (0.02 to 0.41, P < 0.029)	-0.27 (-0.42 to -0.13, P < 0.001)
4	0.0 (0.9)	-0.10 (-0.29 to 0.10, P < 0.333)	-0.58 (-0.72 to -0.44, P < 0.001)
5	-0.1 (0.9)	-0.17 (-0.36 to 0.03, P < 0.090)	-0.66 (-0.80 to -0.52, P < 0.001)
6	-0.3 (0.9)	-0.40 (-0.59 to -0.21, P < 0.001)	-0.83 (-0.97 to -0.68, P < 0.001)
7	-0.4 (0.7)	-0.46 (-0.66 to -0.27, P < 0.001)	-0.88 (-1.03 to -0.74, P < 0.001)
8	-0.5 (0.7)	-0.61 (-0.95 to -0.28, P < 0.001)	-1.04 (-1.28 to -0.80, P < 0.001)
log(milk yield) [-11.3,3.0]	0.0 (1.0)	0.05 (0.01 to 0.09, P < 0.008)	
healthy x log(milk yield) (= ref)			-
clever x log(milk yield)			0.35 (0.24 to 0.47, P < 0.001)
athletic x log(milk yield)			-0.04 (-0.20 to 0.12, P < 0.619)
hypket. x log(milk yield)			0.14 (-0.09 to 0.37, P < 0.248)
PMAS x log(milk yield)			0.20 (0.01 to 0.40, P < 0.043)
week 1. x log(milk yield) (= ref)			-
week 2. x log(milk yield)			-0.11 (-0.23 to 0.02, P < 0.104)
week 3. x log(milk yield)			-0.13 (-0.26 to 0.00, P < 0.069)
week 4. x log(milk yield)			-0.16 (-0.29 to -0.03, P < 0.016)
week 5. x log(milk yield)			-0.22 (-0.35 to -0.08, P < 0.002)
week 6. x log(milk yield)			-0.18 (-0.30 to -0.05, P < 0.007)
week 7. x log(milk yield)			-0.20 (-0.34 to -0.07, P < 0.003)
week 8. x log(milk yield)			-0.30 (-0.53 to -0.08, P < 0.009)

SD: standard deviation, ref: reference, hypket.: hyperketonemic, PMAS: poor metabolic adaptation syndrome, primip.: primiparous, multip.: multiparous

Table 4: Summary of the linear regression for scaled stearic acid (C18:0) concentration in blood (originally in µmol/L blood) with random effects (cow ID, farm ID), observations: n = 2432.

C18:0	mean (SD)	difference in mean values (95% CI, P -value)	coefficient - model (95% CI, P -value)
cowtype			
healthy (= ref)	0.4 (0.8)	-	
clever	-0.4 (0.8)	-0.74 (-0.82 to -0.66, P < 0.001)	
athletic	1.5 (1.0)	1.13 (0.98 to 1.28, P < 0.001)	
hypket.	0.4 (0.8)	-0.00 (-0.20 to 0.19, P < 0.969)	
PMAS	1.3 (0.9)	0.91 (0.71 to 1.10, P < 0.001)	
parity			
multip. (= ref)	-0.0 (1.0)	-	
healthy x multip. (= ref)			-
clever x multip.			-0.67 (-0.75 to -0.59, P < 0.001)
athletic x multip.			0.93 (0.77 to 1.09, P < 0.001)
hypket. x multip.			-0.11 (-0.29 to 0.07, P < 0.223)
PMAS x multip.			0.52 (0.34 to 0.70, P < 0.001)
primip.	0.2 (0.9)	0.20 (0.10 to 0.29, P < 0.001)	0.29 (0.12 to 0.46, P < 0.001)
healthy x primip. (= ref)			-
clever x primip.			-0.51 (-0.65 to -0.37, P < 0.001)
athletic x primip.			0.20 (-0.09 to 0.50, P < 0.171)
hypket. x primip.			-0.25 (-0.88 to 0.38, P < 0.436)
PMAS x primip.			0.61 (0.28 to 0.95, P < 0.001)
week			
1 (= ref)	-0.1 (1.4)	-	-
2	0.5 (1.2)	0.55 (0.35 to 0.75, P < 0.001)	0.15 (0.01 to 0.30, P < 0.045)
3	0.3 (1.0)	0.41 (0.21 to 0.60, P < 0.001)	-0.12 (-0.26 to 0.03, P < 0.119)
4	0.0 (0.9)	0.11 (-0.09 to 0.30, P < 0.283)	-0.41 (-0.56 to -0.27, P < 0.001)
5	-0.0 (0.9)	0.05 (-0.15 to 0.24, P < 0.637)	-0.49 (-0.63 to -0.34, P < 0.001)
6	-0.3 (0.9)	-0.19 (-0.38 to 0.00, P < 0.055)	-0.66 (-0.80 to -0.51, P < 0.001)
7	-0.3 (0.8)	-0.27 (-0.46 to -0.07, P < 0.007)	-0.72 (-0.87 to -0.57, P < 0.001)
8	-0.5 (0.6)	-0.41 (-0.75 to -0.07, P < 0.018)	-0.89 (-1.13 to -0.64, P < 0.001)
log(milk yield) [-11.3,3.0]	-0.0 (1.0)	0.12 (0.08 to 0.16, P < 0.001)	
healthy x log(milk yield) (= ref)			-
clever x log(milk yield)			0.45 (0.34 to 0.57, P < 0.001)
athletic x log(milk yield)			0.05 (-0.11 to 0.22, P < 0.539)
hypket. x log(milk yield)			0.22 (-0.02 to 0.46, P < 0.071)
PMAS x log(milk yield)			0.27 (0.07 to 0.47, P < 0.010)
week 1. x log(milk yield) (= ref)			-
week 2. x log(milk yield)			-0.13 (-0.26 to -0.01, P < 0.050)
week 3. x log(milk yield)			-0.19 (-0.33 to -0.06, P < 0.006)
week 4. x log(milk yield)			-0.22 (-0.35 to -0.09, P < 0.001)
week 5. x log(milk yield)			-0.28 (-0.41 to -0.15, P < 0.001)
week 6. x log(milk yield)			-0.25 (-0.38 to -0.12, P < 0.001)
week 7. x log(milk yield)			-0.26 (-0.40 to -0.13, P < 0.001)
week 8. x log(milk yield)			-0.34 (-0.57 to -0.12, P < 0.004)

SD: standard deviation, ref: reference, hypket.: hyperketonemic, PMAS: poor metabolic adaptation syndrome, primip.: primiparous, multip.: multiparous

Table 5: Summary of the linear regression for scaled oleic acid (C18:1) concentration in blood (originally in µmol/L blood) with random effects (cow ID, farm ID), observations: n = 2432.

C18:1	mean (SD)	difference in mean values (95% CI, P -value)	coefficient – model (95% CI, P -value)
cowtype			
healthy (= ref)	0.5 (0.9)	-	
clever	-0.4 (0.7)	-0.90 (-0.98 to -0.82, P < 0.001)	
athletic	1.6 (1.1)	1.05 (0.91 to 1.20, P < 0.001)	
hypket.	0.1 (0.8)	-0.39 (-0.57 to -0.20, P < 0.001)	
PMAS	1.5 (1.0)	0.95 (0.76 to 1.13, P < 0.001)	
parity			
multip. (= ref)	-0.1 (1.0)	-	
healthy x multip. (= ref)			-
clever x multip.			-0.82 (-0.90 to -0.75, P < 0.001)
athletic x multip.			0.85 (0.69 to 0.10, P < 0.001)
hypket. x multip.			-0.41 (-0.58 to -0.24, P < 0.001)
PMAS x multip.			0.47 (0.30 to 0.64, P < 0.001)
primip.	0.3 (1.0)	0.36 (0.26 to 0.45, P < 0.001)	0.44 (0.28 to 0.60, P < 0.001)
healthy x primip. (= ref)			-
clever x primip.			-0.72 (-0.86 to -0.59, P < 0.001)
athletic x primip.			0.09 (-0.18 to 0.37, P < 0.514)
hypket. x primip.			-0.37 (-0.97 to 0.22, P < 0.220)
PMAS x primip.			0.68 (0.36 to 1.01, P < 0.001)
week			
1 (= ref)	0.0 (1.3)	-	-
2	0.5 (1.1)	0.44 (0.24 to 0.64, P < 0.001)	0.12 (-0.00 to 0.24, P < 0.060)
3	0.3 (1.0)	0.27 (0.08 to 0.47, P < 0.006)	-0.15 (-0.27 to -0.02, P < 0.020)
4	0.0 (0.9)	-0.03 (-0.23 to 0.16, P < 0.756)	-0.44 (-0.57 to -0.32, P < 0.001)
5	-0.1 (0.9)	-0.10 (-0.29 to 0.09, P < 0.318)	-0.53 (-0.66 to -0.41, P < 0.001)
6	-0.3 (0.9)	-0.33 (-0.52 to -0.13, P < 0.001)	-0.68 (-0.81 to -0.56, P < 0.001)
7	-0.3 (0.8)	-0.39 (-0.58 to -0.19, P < 0.001)	-0.75 (-0.87 to -0.62, P < 0.001)
8	-0.5 (0.7)	-0.52 (-0.86 to -0.18, P < 0.003)	-0.93 (-1.15 to -0.72, P < 0.001)
log(milk yield) [-11.3,3.0]	0.0 (1.0)	0.03 (-0.01 to 0.07, P < 0.176)	
healthy x log(milk yield) (= ref)			-
clever x log(milk yield)			0.18 (0.14 to 0.23, P < 0.001)
athletic x log(milk yield)			-0.22 (-0.34 to -0.11, P < 0.001)
hypket. x log(milk yield)			0.02 (-0.19 to 0.22, P < 0.858)
PMAS x log(milk yield)			0.04 (-0.12 to 0.21, P < 0.598)

SD: standard deviation, ref: reference, hypket.: hyperketonemic, PMAS: poor metabolic adaptation syndrome, primip.: primiparous, multip.: multiparous

Figure 1: Effect plots of the linear model for scaled palmitic acid (C16:0) concentration in blood (n = 2432 observations , originally in µmol/L blood) with 95% confidence intervals. Effects: (a) parity, (b) log(milk yield) and (c) week x log(milk yield), originally in L/d. The cow types are as following: (1): healthy, (2): clever, (3): athletic, (4): hyperketonemic and (5): poor metabolic adaptation syndrome (PMAS).

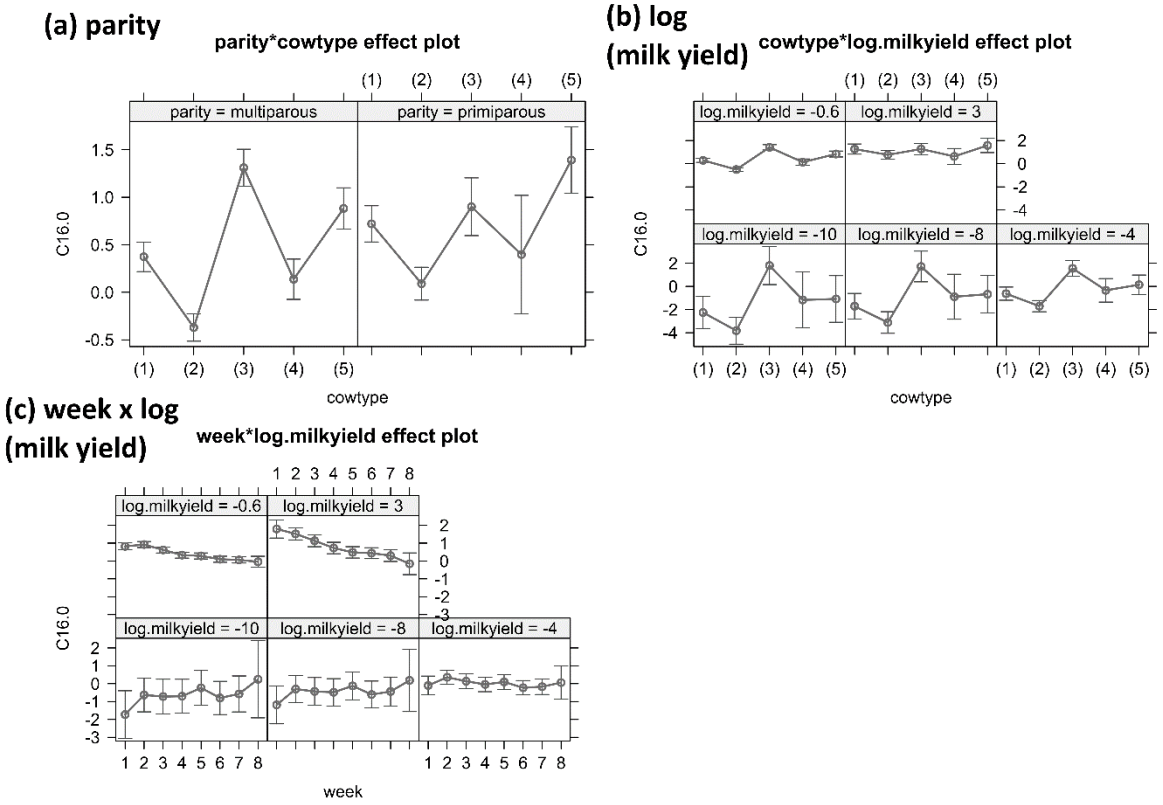


Figure 2: Effect plots of the linear model for scaled stearic acid (C18:0) concentration in blood (n = 2432 observations , originally in µmol/L blood) with 95% confidence intervals. Effects: (a) parity, (b) log(milk yield) and (c) week x log(milk yield), originally in L/d. The cow types are as following: (1): healthy, (2): clever, (3): athletic, (4): hyperketonemic and (5): poor metabolic adaptation syndrome (PMAS).

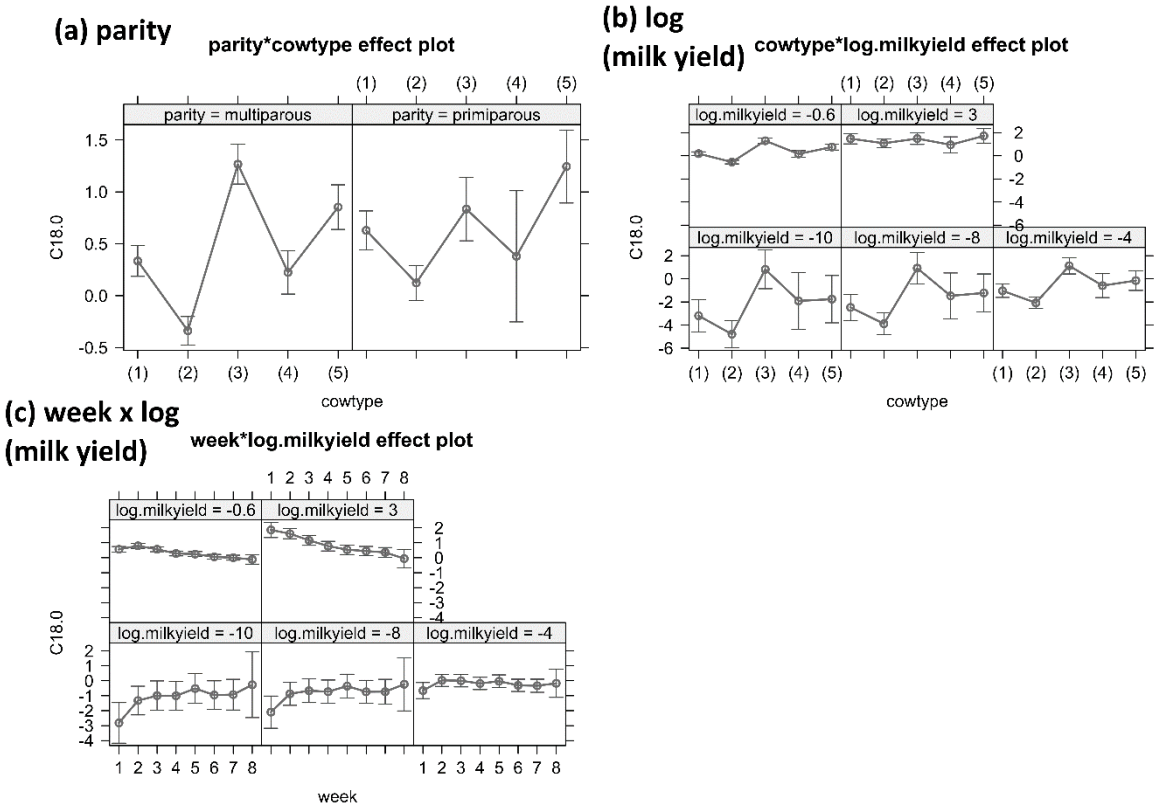
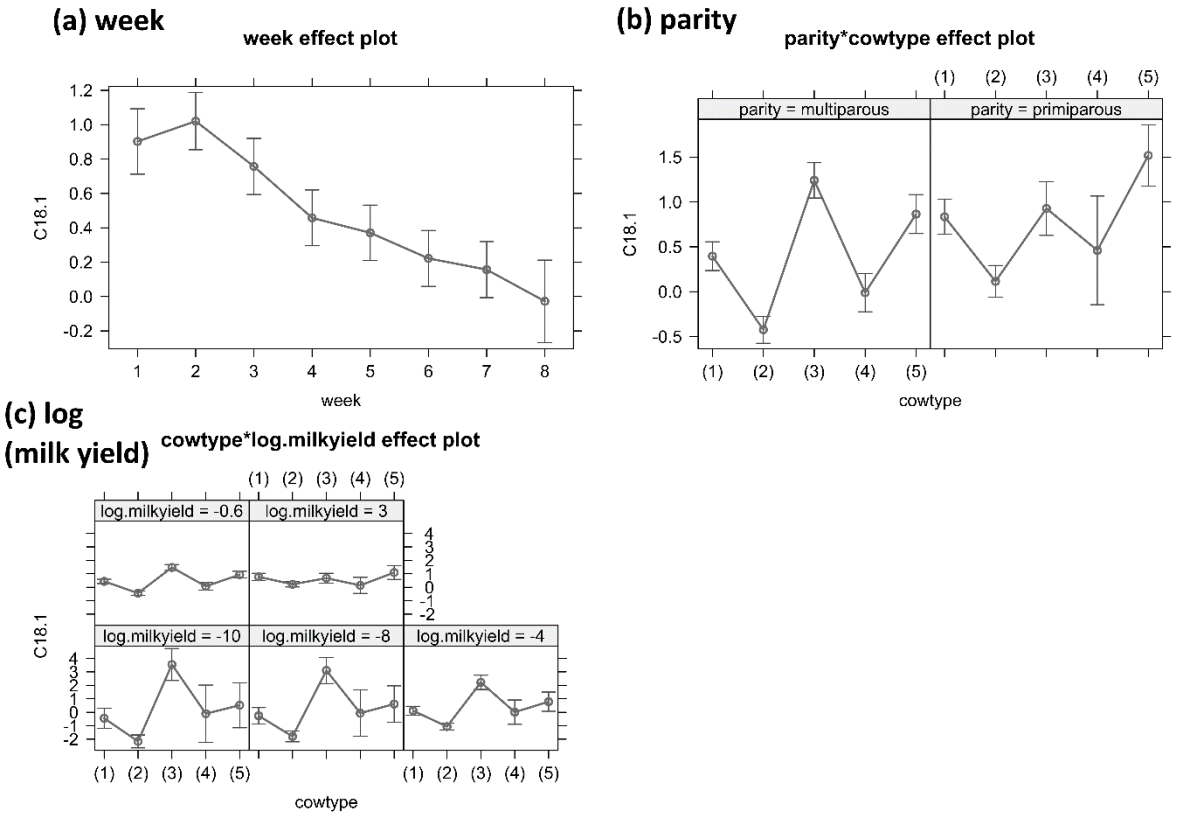


Figure 3: Effect plots of the linear model for scaled oleic acid (C18:1) concentration in blood (n = 2432 observations , originally in µmol/L blood) with 95% confidence intervals. Effects: (a) week, (b) parity and (c) log(milk yield), originally in L/d. The cow types are as following: (1): healthy, (2): clever, (3): athletic, (4): hyperketonemic and (5): poor metabolic adaptation syndrome (PMAS).



V. DISCUSSION

1. Examined Fatty Acids and Effects

When comparing blood and milk FA concentrations and their differences between cow types, it becomes noticeable that all three examined FA concentrations show the same differences between cow types for blood FA but not for milk FA. For all examined blood FA concentrations clever cows show the lowest concentration, followed by hyperketonemic, healthy and PMAS cows and with the athletic cow type showing the highest concentration. The same order was found for the milk C18:1 concentration and similar for milk C18:0 concentration, where athletic cows showed the highest and PMAS cows the second highest concentration. Milk C16:0 concentrations showed a different order with hyperketonemic cows having the lowest concentration, followed by clever, athletic and PMAS cows and healthy cows showing the highest concentration. These findings might be explained by the fact that milk C18:0 and C18:1 FA origin from blood FA and are increased during a NEB (Bauman and Griinari, 2003), and therefore their blood and milk concentrations are expected to be similar within the same cow type representing a metabolic health status. Milk C16:0 on the other hand can be both de novo synthesized while the milk is produced or origin from the blood (Bauman and Griinari, 2003). This is in accordance with differing concentrations of blood and milk FA between the cow types.

When considering effects and interaction terms, C18:1 concentrations also show great similarities between blood and milk. For C18:0 concentrations, the effects and interaction terms are also quite similar apart from hyperketonemic cows showing relatively higher concentrations when compared to healthy cows for multiparous cows and within lower milk yield in blood than in milk. This is in accordance with the findings discussed before (Bauman and Griinari, 2003).

Effects and interaction terms found for blood C16:0 concentrations vary notably from those for milk C16:0 concentrations. For example, blood C16:0 concentrations increase with increasing milk yield while milk C16:0 concentrations decrease with increasing milk yield. This finding can also be explained with the fact that milk C16:0 is both synthesized de novo and derived from blood C16:0 and compared to longer chained FA decreases with an increasing metabolic challenge, such as a higher milk yield (Bauman and Griinari, 2003).

2. Previous Use of the Blood and Milk Fatty Acids

Previous research focused on the gain of knowledge through the examination of FA concentrations and ratios in milk (Reus and Mansfeld, 2020). It can be seen as the base for application in herd health monitoring.

Not much research was carried out on the FA composition in blood. This may be due to the lack of use. The concentration of NEFA in blood can be and is reliably used to determine a metabolic disorder (Tremblay et al., 2018). Though information can be obtained by examining the FA composition, there is little prospect in clinical use that the NEFA concentration cannot give, as the amount of work is at least the same (collecting blood samples and sending them to a laboratory).

Earlier research and use of milk FA analysis focused on the analysis via gas chromatography (Pedron et al., 1993; Van Haelst et al., 2008; Jorjong et al., 2014). The analysis using gas chromatography is very exact and allows to differentiate between various FA. It is a time and work consuming process, which is very useful for research, but is not practicable for routine and herd-based milk analysis. This research focused on identifying single FA, FA groups [e.g., conjugated linolenic acid (CLA) or short-chained FA (SCFA)] or FA ratios that can be used as a marker for metabolic diseases by defining a threshold concentration. Useful findings were made, and some thresholds are promising in the detection of diseased animals in high-throughput milk analysis, such as FTIR.

More recent research focused on data obtained through high-throughput milk analysis (Bach et al., 2019; Mantysaari et al., 2019). As a much bigger amount of data is gained, it becomes more and more important to identify relevant FA or to effectively process the available data. The downside, that measurements are not as exact as via gas chromatography, can be outweighed by the amount of data that is produced and available for processing. The focus of the research is on the wise use of methods on data processing. Interesting models for the prediction of increased blood BHBA or NEFA values have been calculated (Tremblay et al., 2019). They offer the potential to be valuable in herd health monitoring.

3. Use of the Blood and Milk Fatty Acids in this Work

The present work focuses on the determination of cow types and especially the description of differences between them considering blood and milk FA concentrations. Cows have different mechanisms in adapting or failing to adapt to the NEB physiologically occurring after

parturition (Tremblay et al., 2018; Mandujano Reyes et al., 2023). Milk FA have been analyzed in various ways and with various objectives in recent studies, yet not much research with the aim of understanding the FA profile changes in blood FA was carried out. Instead, blood NEFA concentration is widely used and becoming more important as a reference for a metabolic disorder (Tremblay et al., 2018). One objective of this work was to deeper understand the mechanisms of adaptation or failure to adapt by comparing the blood and milk FA concentrations within the different cow types regarding the difference between heifers and cows, over time after parturition and differences in milk yield.

Usually, blood FA concentrations are determined by analyzing blood samples. Within this work, blood FA concentrations were calculated using milk FTIR data and applying a regression model. This implies that for the use of calculated blood FA concentrations, no blood sample is needed, and it could therefore allow the use within high-throughput milk analysis. The differences between blood and milk FA concentrations regarding cow types were described and discussed earlier. Especially within C16:0 marked differences between blood and milk FA can be found. This supports the thesis, that blood FA concentrations add information on the metabolic health status to using milk FA concentrations only, even though being calculated from milk FTIR data. As there were no reference blood FA concentrations analyzed in blood samples, it is one limitation of this work, that the information gained from calculated blood FA concentrations cannot be compared to blood FA concentrations analyzed in blood samples.

Interestingly, effects and interaction terms are quite similar between the examined blood FA concentrations, especially between C18:0 and C18:1. This finding supports the thesis, that the changes in concentration are rather uniform between the different blood FA. Which in turn justifies the established use of NEFA as a general marker (Tremblay et al., 2018) and the lack of research within blood FA. Nevertheless, different FA concentrations behave slightly different within the same metabolic status, according to whether they are derived from mobilized adipose tissue or not. The described findings are partly explained using the unit µmol/L blood, while milk FA are measured in general in g/100 g fat. This implies, that concentration changes in relation to other FA are easier to detect in milk compared to blood due to the used unit.

Considering milk FA concentrations, they are not as similar between the different FA concentrations as blood FA. As already discussed, this is in accordance with the expectation, that the milk of metabolically challenged cows contains more preformed and less de novo synthesized FA (Bauman and Griinari, 2003).

Both blood and milk FA concentrations show marked differences within the cow types and the different examined effects, especially between the PMAS cow type and the other cow types, but also between the other cow types at a closer look. Therefore, the focus should be on the differentiation between cow types, additionally using the examined effects. The cow types reflect the different possible reactions to the physiologically occurring NEB. Cow types can be distinguished using milk FA concentrations analyzed with FTIR and as a result can be managed differently.

4. Application of the Results and Outlook

Challenges of modern dairy farming including the increased average and single cow's milk yield need a constantly improving and more detailed monitoring of the cows during the risk period around calving (Gruber et al., 2021). Detecting cows of the cow types at risk and determining the cow type will help to improve the understanding and care for the cow's needs. By considering the whole herd and the proportion of each cow type compared to the herd, it can be responded to the demands of the whole herd.

Furthermore, evaluating the results of routine milk analysis is often underrated and neglected and great potential is lost (Reiter et al., 2021). The results of the study can potentially address this challenge, as they can lead to a refined output for the farmer that could facilitate the interpretation as also suggested by other authors (Hajek et al., 2023) and the required reaction.

Another challenge that needs to be addressed is the limited potential due to the currently only monthly analysis. Smaller intervals between the sample collection should be considered (Gruber et al., 2021).

5. Conclusion

The use of blood and milk FA concentrations offers great potential in the monitoring of dairy herds' health. Studies have shown the association between certain FA concentrations or FA ratios surpassing a defined threshold and signs for metabolic disorders, such as hyperketonemia or elevated blood NEFA concentrations (Reus and Mansfeld, 2020). The classification of cows into the five cow types healthy, clever, athletic, hyperketonemic and PMAS allows a more detailed consideration of the cow, both on a herd and an individual cow level. Each cow type's different behavior of FA concentrations within the variables parity, milk yield and DIM can

help to differentiate between the cow types and to determine the proportion of each cow type compared to the whole herd. Cows can be managed according to their cow type, assuring their needs are met as adequately as possible.

VI. SUMMARY

Major challenges in modern dairy farming are increasing (milk) yield while the cow's physiology remains unchanged. Increasing productivity can decrease the health status of both the individual cow and on the herd level. At the same time, the possibilities of monitoring the herd health status of cows have never been higher due to increasingly accessible and extensive data collection.

Metabolic disorders play a big role in the cows' health, e.g., ketosis with a reported prevalence of 8 – 22% and incidence of 16 – 43%. The newly described term "Poor Metabolic Adaptation Syndrome (PMAS)" puts the focus on the (lacking) ability of the cow to adapt to the physiologically occurring negative energy balance after parturition instead of on the concentration of ketone bodies in blood, quantified by using the ß-hydroxybutyrate acid (BHBA) concentration in blood. PMAS is mainly characterized by the concentration of non-esterified fatty acids (NEFA) in blood.

Together with the established cow-side tests using blood, urine, or milk that have the disadvantage of a relatively high (time) effort, Fourier-transform infrared (FTIR) analysis of milk in high-throughput technologies is a promising approach. This dissertation investigated the correlation between blood and milk FA concentrations and metabolic disorders.

The first publication is a review describing the possibility of using milk FA for predicting metabolic disorders in dairy cows. Ten studies were included; three examined the correlation between milk FA and NEFA in blood, three the correlation between milk FA and both NEFA and BHBA in blood, and four the correlation between milk FA and BHBA in blood. Decreased concentrations of short and medium-chained FA (C4 – C14 and C5 – C15) were associated with metabolic disorders, while concentrations of long-chained FA as cis-9 C18:1 were increased during a metabolic disorder. Some FA concentrations, such as cis-9 C16:1, and FA ratios 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. The analysis of correlation coefficients suggests that specific ratios might be useful for herd health monitoring. Two studies developed linear regression models using FA concentrations, FA ratios, and further information to predict the metabolic health status. Refined models to predict the health status of individual cows and the whole herd might be more promising than using single FA or FA ratios to detect cows suffering from metabolic

disorders. These prediction models can potentially become a supporting tool in routine herd health monitoring.

The second publication focuses in detail on the milk FA concentrations as well as other associated factors for different metabolic health statuses. During weekly visits on n = 8 farms over 51 weeks, blood samples were collected from Simmental cows between 5 and 50 days in milk (DIM). The farmer collected corresponding milk samples. Milk FA concentrations were determined using milk FTIR data, while blood FA concentrations were calculated using milk FTIR data and applying a linear regression model. N = 2432 observations from n = 553 Simmental cows were used for the analysis. The observations were assigned to five different cow types (healthy, clever, athletic, hyperketonemic, and PMAS), representing five metabolic health statuses. The classification is based on the thresholds of 1.2 mmol/L, 0.7 mmol/L, and 1.4 for the concentrations of ß-hydroxybutyrate acid, nonesterified fatty acids, and milk fat-to-protein ratio, respectively. Linear regression models using the predictor variables cow type, parity, week of lactation, and milk yield as fixed effects and interaction terms were developed to test for significant associations with the outcome variables FA concentrations in blood and milk. There was a significant interaction term found between PMAS cows and parity compared to healthy cows for milk C18:1 ($P < 0.001$) and for milk C18:0 ($P < 0.01$). It revealed higher concentrations for PMAS in primiparous and multiparous cows compared to healthy cows, the slope between PMAS and healthy cows being steeper for primiparous cows than for multiparous cows. Further, an interaction term was found between PMAS cows and milk yield compared to healthy cows and milk yield for milk C16:0 ($P < 0.05$), revealing a steeper slope for decreasing C16:0 concentrations with increasing milk yield for PMAS compared to healthy cows.

When considering effects and interaction terms, C18:0 and C18:1 concentrations show remarkable similarities between blood and milk. On the contrary, effects and interaction terms for blood C16:0 concentrations deviate notably from those for milk C16:0 concentrations. For example, blood C16:0 concentrations increase with increasing milk yield, while milk C16:0 concentrations decrease with increasing milk yield. This emphasizes that blood FA concentrations add information on metabolic health status compared to using milk FA concentrations only.

The associations and interaction terms between cow type, parity, week, and milk yield as predictor variables and blood and milk FA concentrations as outcome variables suggest

different dimensions of management practices. They could identify cows at risk on both the herd and the individual cow level and optimize sustainable productivity and welfare.

Evaluating the results of routine milk analysis is often underrated, leaving great potential unused. The study results could increase the use by creating a refined output by assigning the cows to the cow types and facilitate the interpretation and the required reaction to the respective metabolic health status. Furthermore, shorter intervals for milk analysis should be considered to improve the quality of herd health monitoring.

VII. ZUSAMMENFASSUNG

Steigende (Milch-)Leistungen trotz unveränderter Physiologie sind aktuelle Herausforderungen in der Milchviehhaltung. Höhere Leistungen können mit einer schlechteren Gesundheit der einzelnen Kuh, sowie der Herde einhergehen. In der heutigen Zeit bietet eine fortschreitende Digitalisierung immer mehr Möglichkeiten, durch automatisierte, umfassende und daher vereinfachte Datenerhebung, den Gesundheitsstatus von Milchkühen zu erfassen.

Stoffwechselerkrankungen sind eine große Herausforderung der Kuhgesundheit im postpartalen Zeitraum. Hier ist vor allem die Ketose mit beschriebenen Prävalenzen von 8 – 22% sowie Inzidenzen von 16 – 43% zu nennen. Das neu beschriebene „Poor Metabolic Adaptation Syndrome (PMAS)“ betont dabei weniger die tatsächliche Ketonkörper-konzentration im Blut, ausgedrückt durch die ß-Hydroxybuttersäure (BHBA)-Konzentration im Blut, sondern die (mangelnde) Fähigkeit der Kuh sich an den physiologisch nach der Kalbung auftretenden Energiemangel anzupassen. PMAS ist vor allem durch die Konzentration an freien Fettsäuren (non-esterified fatty acids, NEFA) im Blut charakterisiert.

Bereits etablierte Schnelltests, die im Stall mit Blut, Urin oder Milch durchgeführt werden können, sind sehr zeitaufwendig. Ein vielversprechender Ansatz im Herdenmonitoring ist die Fourier-Transform-Infrarot (FTIR) Analyse der Milch im Hochdurchsatzverfahren. In dieser Arbeit wurde der Zusammenhang zwischen Blut- und Milchfettsäurekonzentrationen einerseits und Stoffwechselstörungen andererseits untersucht.

Die erste Veröffentlichung ist ein Übersichtsartikel und beschreibt die Möglichkeiten der Vorhersage von Stoffwechselstörungen bei Milchkühen anhand von Milchfettsäurekonzen-trationen. Es wurden zehn Veröffentlichungen verglichen, wovon drei die Korrelationen zwischen Milchfettsäureprofilen und NEFA im Blut untersuchten, drei zwischen Milchfettsäureprofilen und sowohl NEFA als auch BHBA im Blut, und vier zwischen Milchfettsäureprofilen und BHBA im Blut. Niedrigere Konzentrationen von kurz- und mittelkettigen Fettsäuren (C4 – C14 und C5 – C15) waren mit Stoffwechselstörungen assoziiert, während langkettige Fettsäuren, vor allem cis-9 C18:1, höhere Konzentrationen während einer Stoffwechselstörung zeigten. Einige Fettsäurekonzentrationen, wie cis-9 C16:1, sowie die Fettsäurequotienten cis-9 C16:1/C15:0, C17:0/C15:0 und C18:1/C15:0 korrelierten ebenfalls mit einer Stoffwechselstörung. Eine Analyse der Korrelationskoeffizienten zeigt, dass eine Nutzung im Herdenmonitoring möglich sein könnte. Neue lineare Regressionsmodelle

basierend auf Fettsäurekonzentrationen, Fettsäurequotienten und weiteren Informationen wurden entwickelt, um den Stoffwechselstatus vorherzusagen. Die Nutzung von optimierten Vorhersagemodellen zum Stoffwechselstatus könnte zielführender sein als die Nutzung von einzelnen Fettsäurekonzentrationen oder Fett-säurequotienten. Diese Vorhersagemodelle könnten im Herdenmonitoring genutzt werden.

In der zweiten Veröffentlichung werden die Veränderungen der Blut- und Milchfettsäureprofile, sowie assoziierte Faktoren bei unterschiedlichem Stoffwechselstatus untersucht. Bei 51 wöchentlichen Bestandsbesuchen auf n = 8 Betrieben wurden Blutproben von Fleckviehkühen zwischen 5 und 50 Tagen in Milch (days in milk, DIM) genommen. Der jeweilige Betriebsleiter nahm dazugehörige Milchproben. Milchfettsäurekonzentrationen wurden anhand von Milch FTIR-Daten bestimmt. Blutfettsäurekonzentrationen wurden ebenfalls anhand von Milch FTIR-Daten bestimmt, welche durch die Anwendung von linearen Regressionsmodellen berechnet wurden. N = 2750 Proben, bestehend aus einer Blut- und zugehörigen Milchprobe, von n = 553 Kühen wurden analysiert. Jede Beobachtung wurde einer von fünf Kuhtypen zugewiesen (Gesund, Clever, Athletisch, Hyperketonämisch und PMAS) welche jeweils einem Stoffwechselstatus entspricht. Die Einteilung wurde anhand der Grenzwerte 1,2 mmol/L BHBA, 0,7 mmol/L NEFA und 1,4 für den Fett-Eiweiß-Quotient durchgeführt. Lineare Regressionsmodelle, basierend auf den Vorhersagevariablen Kuhtyp, Laktationszahl, Woche in Laktation und Milchleistung als feste Effekte und Interaktionsterme, wurden entwickelt und auf signifikante Assoziationen mit der jeweiligen Ergebnisvariable Fettsäurekonzentration in Blut und Milch getestet. Ein signifikanter Interaktionsterm wurde zwischen dem PMAS Kuhtyp und Laktationszahl im Vergleich zum gesunden Kuhtyp für Milch C18:1 ($P < 0,001$) und für Milch C18:0 ($P < 0,01$) gefunden. Hier zeigten sich höhere Konzentrationen im PMAS Kuhtyp für Kalbinnen und multipare Kühe im Vergleich zum gesunden Kuhtyp, mit einem ausgeprägteren Gefälle zwischen PMAS und gesunden Kühen bei Kalbinnen als bei multiparen Kühen. Weiterhin gab es einen Interaktionsterm zwischen dem PMAS Kuhtyp und Milchleistung im Vergleich zum gesunden Kuhtyp für Milch C16:0 Konzentrationen ($P < 0,05$). Dieser zeigte einen steileren Abfall der C16:0 Konzentrationen mit steigender Milchleistung für den PMAS Kuhtyp im Vergleich zum gesunden Kuhtyp.

Feste Effekte und Interaktionsterme zeigen große Ähnlichkeiten zwischen Blut- und Milchfettsäurekonzentrationen für C18:0 und C18:1, während diese sich bei C16:0 deutlich voneinander unterscheiden. Zum Beispiel steigen C16:0 Blutfettsäurekonzentrationen mit steigender Milchleistung, während diese Konzentrationen bei steigender Milchleistung in der

Milch sinken. Blutfettsäurekonzentrationen ergänzen also die durch Milchfettsäurekonzentrationen verfügbaren Informationen zum Stoffwechselstatus.

Die Assoziationen und Interaktionsterme zwischen den Vohersagevariablen Kuhtyp, Laktationszahl, Woche in Milch und Milchleistung und den Ergebnisvariablen Fettsäurekonzentrationen (jeweils in Blut und Milch) deuten darauf hin, dass die verschiedenen Kuhtypen im Herdenmanagement individuell berücksichtigt werden sollten. Sowohl auf Einzeltier- als auch auf Herdenebene könnten Problemtiere und -gruppen identifiziert und so eine nachhaltige Wirtschaftlichkeit und das Tierwohl gefördert werden.

Die Ergebnisse der regelmäßigen Routine-Milchuntersuchung werden oft nicht adäquat genutzt. Hierdurch bleibt eine vielversprechende Möglichkeit im Herdenmonitoring ungenutzt. Die Ergebnisse der Studie könnten dazu beitragen, diese Informationen gezielter zu verwenden, indem sie durch die Einteilung der Kühe in die Kuhtypen den direkten Nutzen der Informationen erhöhen und so Handlungsanpassungen an den jeweiligen Stoffwechselstatus vereinfachen könnten. Weiterhin sollten kürzere Milchuntersuchungsabstände angeboten werden, um die Qualität des Herdenmonitorings zu verbessern.

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