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# Ketosis risk derived from mid-infrared predicted traits and its relationship with herd milk yield, health and fertility

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Milk analysis using mid-infrared spectroscopy (MIR) is a fast and inexpensive way of examining milk samples on a large scale for fat, protein, lactose, urea and many other novel traits. A new indicator trait for ketosis, KetoMIR, which is based on clinical ketosis diagnoses and MIR-predicted traits, was developed by the Regional State Association for Performance and Quality Inspection in Animal Breeding of Baden Württemberg in 2015. The KetoMIR result is available for each cow at milk recording during the first 120 days in milk and presented to farmers in three classes: 1 = low ketosis risk, 2 = moderate ketosis risk and 3 = high ketosis risk. The aim of the current study was to analyze the phenotypic relationships between KetoMIR and milk yield, fertility and health at the herd level. Annual herd reports from 12,909 herds with an average herd size of 27 cows were available for the analyses. Overall, the mean incidence of ketosis (KetoMIR risk class 2 or 3) at the herd level was 14.0%. Farms with the lowest ketosis risk ( $\leq 10\%$  of cows in the herd with a moderate or high ketosis risk) differed in all variables from the farms with the highest ketosis risk ( $> 50\%$  of cows in the herd with a moderate or high ketosis risk). The increased ketosis risk based on KetoMIR was associated with lower average herd milk yield (-1,975 kg milk). Mean herd somatic cell count in first and higher lactations was increased by 60,500 and 134,400 cells/ml, respectively. The interval from calving to first service was prolonged by +36.5 days, as was the calving interval with +58.2 days. The newly developed KetoMIR trait may be used in ketosis prevention programs.

## KEYWORDS

ketosis, mid infrared spectra, milk components, herd management, dairy cattle

## Introduction

Ketosis is one of the most common metabolic diseases of dairy cows and occurs at the beginning of lactation. The incidence of clinical ketosis is low and is only 1.3% in Austria (Fürst-Walzl and Schwarzenbacher, 2021). Subclinical ketosis occurs much more frequently but is not routinely recorded. Egger-Danner et al. (2016) and Liebinger (2021) reported that 10 to 20% of cows in Austria are affected with subclinical ketosis. The range of ketosis incidence differs for each country, as for example in the Netherlands, much higher values were reported by Vanholder et al. (2015) in 23 dairy farms, 11.6% of cows had clinical ketosis and 47.2% had subclinical ketosis. Lean et al. (2023) conducted a retrospective meta-analysis based on individual cow data from Australia, Canada and the United States. They reported the incidence of clinical ketosis was 3.3%, whereas the incidence of subclinical ketosis was 26.8%.

Ketosis can be diagnosed by measuring ketone bodies present in blood, urine, or milk. Various handheld blood ketone meters are available to farmers to detect cows with subclinical ketosis (e.g. Sailer et al., 2018; Khol et al., 2019). However, due to practical limitations associated with individual blood sampling and testing, routine screening of all at-risk animals with blood tests is not feasible. Analysis of individual milk samples by mid-infrared (MIR) spectrometry remains the most promising method for detecting ketosis because it allows complete screening of the health status of the animal and the herd (Benedet et al., 2019). In recent years, many novel MIR-predicted traits related to metabolic health have been derived (de Roos et al., 2007; Grelet et al., 2016; Franceschini et al., 2022). A new ketosis risk indicator trait, KetoMIR, based on clinical ketosis diagnoses and MIR-predicted traits, was developed at the Regional State Association for Performance and Quality Inspection in Animal Breeding of Baden Württemberg as part of the OptiMIR project (Hamann et al., 2017; Drössler et al., 2018). The new ketosis risk indicator is intended as a herd management tool that allows farmers to monitor the metabolic situation of their herd during the first 120 days of lactation. In Baden-Württemberg, KetoMIR has been routinely used since 2015, and in Austria since 2017. The aim of the present study was to investigate the relationship of ketosis risk based on KetoMIR with milk yield, health and fertility at herd level in Austrian dairy farms. For this purpose, the phenotypic associations between these traits were analyzed.

## Materials and methods

### Description of KetoMIR

The trait KetoMIR was developed at the Regional State Association for Performance and Quality Inspection in Animal Breeding of Baden Württemberg as part of the OptiMIR project (Hamann et al., 2017; Drössler et al., 2018). The new trait was derived based on clinical ketosis diagnoses and MIR-predicted traits. The model used is a logistic regression and includes nine

MIR-predicted traits to predict ketosis risk, including standard milk components, ketone bodies, fatty acids and minerals. A more detailed description of the MIR-predicted traits included in the model cannot be given for reasons of confidentiality. Lactation number, lactation week, breed and sampling time (mixed, morning, evening) are included as fixed effects in the model. The result from the logistic regression model is a continuous value between 0 and 1. The KetoMIR result is available for each cow at milk recording during the first 120 days in milk and is presented to farmers in three classes. Class 1 indicates low ketosis risk (0-0.5), class 2 indicates moderate ketosis risk (>0.5-0.75), and class 3 indicates high ketosis risk (>0.75). A more detailed description of the model can be found in Hamann et al. (2017) and Drössler et al. (2018).

### Data

Annual herd reports from 2017 were provided by ZuchtData (Vienna, Austria), which included herd reports from all Austrian dairy herds under milk recording (in total 21,342 herds). Herds with less than 10 cows and missing data were deleted, which resulted in 12,909 herds. For analyses, herd reports from 12,909 herds with an average herd size of 27 cows were available.

The annual herd reports provide the basis for evaluating the general performance, health and fertility of the herds. The following herd variables were available in the reports: herd means for milk yield, somatic cell count, interval from calving to first insemination and calving interval (see a more detailed description in Table 1). Additionally, the proportion of test-day records with a moderate or high ketosis risk (KetoMIR risk class 2 or 3) within the first 120 days in milk was available for each herd for the year 2017. A summary statistic of the analyzed traits is given in Table 1.

### Statistical analyses

All analyses are based on the herd reports from 2017, and are, therefore, taken from herd mean information and not individual cow data. The analyses were carried out across breeds. The main dairy cattle breed in Austria is Fleckvieh (74.7%), followed by Holstein (7.4%) and Brown Swiss (5.6%) (RZA Annual Report, 2022). A separate analysis for each breed was not possible as many herds have more than one breed or crossbred cows and the herd means for milk yield, somatic cell count, interval from calving to first insemination and calving interval are routinely calculated for each herd across all animals (without taking the breed into account).

All statistics were calculated using SAS software (SAS Institute Inc.). The farms were assigned to one of the following 6 groups according to the proportion of cows in the herd with a moderate or high risk of ketosis (KetoMIR risk class 2 or 3):  $\leq 10$ ,  $>10-\leq 20$ ,  $>20-\leq 30$ ,  $>30-\leq 40$ ,  $>40-\leq 50$ ,  $>50\%$ . The Kruskal-Wallis nonparametric test was used to determine whether there were significant differences between the groups (Kruskal and Wallis, 1952). For multiple comparison, the Dwass, Steel, Critchlow-Fligner multiple

**TABLE 1** Definition of herd variables in Austrian annual herd reports, number of herds and herd mean values for KetoMIR (% of cows in the herd with a moderate or high ketosis risk), milk yield, somatic cell count, calving-to-first-service-interval and calving interval.

Herd variables	Definition	N	Herd mean
KetoMIR	% of test-day records with a moderate or high ketosis risk (5-120 DIM), %	12,909	14.0
Milk yield	Mean herd milk yield (kg)	12,909	7,405
Somatic cell count-First lactation	Mean somatic cell count in cells/mL ( $\times 1,000$ )	12,909	102.3
Somatic cell count-Second and higher lactations	Mean somatic cell count in cells/mL ( $\times 1,000$ )	12,909	188.2
Calving-to-first-service interval	Mean days from calving to first insemination	12,841	75.8
Calving interval	Mean days between the previous and current calving	12,906	387.4

comparison procedure was applied if the p-value was lower than 0.05 for the global null hypothesis (Critchlow and Fliener, 1991).

## Results

In our study, the mean incidence of cows with a moderate or high ketosis risk based on KetoMIR was 14% at the herd level (Table 1). Highly elevated incidences of >30% were found in 8.6% of farms (Figure 1).

The Kruskal-Wallis test determined that there are significant differences in all investigated variables between farms at different risk of ketosis. The subsequently-applied Dwass, Steel, Critchlow-Fliener multiple comparison procedure showed that the farms with the lowest ketosis risk ( $\leq 10\%$  of cows in the herd with a moderate or high ketosis risk) differ in all variables from the farms with the highest ketosis risk (>50% of cows in the herd with a moderate or high ketosis risk) (Table 2). The increased ketosis risk based on KetoMIR was associated with lower average herd milk yield (-1,975 kg milk). Mean herd somatic cell count in first and higher lactations was increased by 60,500 and 134,400 cells/ml, respectively. The interval from calving to first service was prolonged by +36.5 days, as was the calving interval with +58.2 days.

## Discussion

The reported incidence of ketosis based on KetoMIR in Austria is at the lower end of the range published in the literature for dairy cattle. A study in 10 European countries indicated that the overall prevalence of ketosis, defined as serum  $\beta$ -hydroxybutyrate concentration  $\geq 1,200$  to 1,400  $\mu\text{mol/L}$  in cows within 2 to 15 days in milk was 21.8%, ranging from 11.2 to 36.6% (Suthar et al., 2013). Berge and Vertenten (2014)

reported that 39% of the cows in western European dairy herds were classified as having ketosis, defined as a milk based keto test result  $\geq 100 \mu\text{mol/L}$ . The herd average of ketosis was 43% in Germany, 53% in France, 31% in Italy, 46% in the Netherlands, and 31% in the United Kingdom. Of the 131 investigated farms, 112 (85%) had 25% or more of their fresh cows resulting as positive for ketosis. Brunner et al. (2019) reported prevalence of subclinical ketosis ( $\beta$ -hydroxybutyrate concentration  $\geq 1.2 \text{ mmol/L}$ ) in dairy cows in Central and South America, Africa, Asia, Australia, New Zealand, and Eastern Europe. Across all investigated countries, the prevalence of subclinical ketosis was 24.1%, ranging from 8.3% up to 40.1%.

In our study, a higher incidence of cows at moderate or high risk of ketosis on the farm was associated with lower milk yield, higher somatic cell count, longer time from calving to first service and prolonged calving interval, which is in agreement with previous studies. A negative association between the herd prevalence of ketosis and milk yield was also recently reported by Couto Serrenho et al. (2023). As mentioned by Daros et al. (2022) it is likely that herds that produce more milk also have better general management and thus may have implemented more effective ketosis prevention protocols.

In agreement with our results, van Straten et al. (2009) reported that cows with ketosis were 44% more likely to have an event with a somatic cell count >250,000 cells/ml than cows without ketosis. In addition, it was found that cows with extreme relative body weight loss in early lactation were more likely to have an increased somatic cell count throughout lactation.

An undesirable relationship of ketosis and fertility was reported by numerous authors (Reist et al., 2000; Walsh et al., 2007; Rutherford et al., 2016). Compton et al. (2015) found that concentration of  $\beta$ -hydroxybutyrate in blood  $\geq 1.2 \text{ mmol/L}$  at any stage within 5 weeks post-calving was associated with decreased 6-week pregnancy rate (78 vs. 85%). Recently, Alemu et al. (2023) reported a negative association between elevated milk  $\beta$ -hydroxybutyrate within 42 d and reproductive performance after the voluntary waiting period.

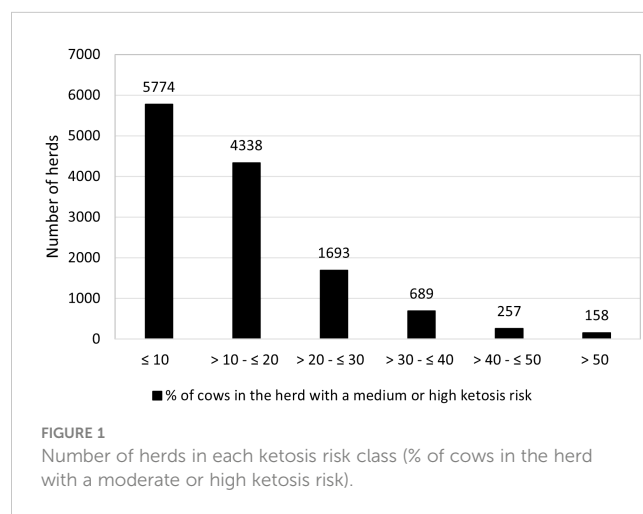


TABLE 2 Herd mean values, median, 25<sup>th</sup> percentile and 75<sup>th</sup> percentile for milk yield, somatic cell count, calving-to-first-service-interval and calving interval with respect to ketosis risk (% of cows in the herd with a moderate or high ketosis risk according to KetoMIR result).

% of cows in the herd with a moderate or high risk of ketosis	Trait	Herd mean	25 <sup>th</sup> percentile	Median	75 <sup>th</sup> percentile	Significance <sup>1</sup>
≤10	Milk yield, kg	7,765	6,836	7,718	8,651	a
>10-≤20		7,379	6,380	7,305	8,270	b
>20-≤30		6,937	5,975	6,820	7,799	c
>30-≤40		6,549	5,561	6,490	7,514	d
>40-≤50		6,133	5,348	6,078	6,922	e
>50		5,790	4,829	5,863	6,750	e
≤10	Somatic cell count-First lactation, cells/mL (×1,000)	92.5	49.9	74.5	114.7	a
>10-≤20		104.5	54.6	81.6	126.2	b
>20-≤30		114.3	58.4	88.8	141.8	c
>30-≤40		121.8	58.7	92.2	143.2	c
>40-≤50		125.8	59.5	102.9	163.3	cd
>50		153.0	69.2	100.6	190.8	d
≤10	Somatic cell count-Second and higher lactations, cells/mL (×1,000)	160.6	97.2	143.6	203.2	a
>10-≤20		197.5	121.6	174.1	247.6	b
>20-≤30		218.5	134.7	193.6	275.2	c
>30-≤40		235.7	144.1	209.0	285.1	cd
>40-≤50		258.2	147.9	223.4	327.5	de
>50		295.0	177.0	257.7	357.9	e
≤10	Calving-to-first-service interval, days	71.7	59.8	67.9	78.7	a
>10-≤20		75.0	61.9	70.8	82.8	b
>20-≤30		81.8	64.9	76.2	91.6	c
>30-≤40		87.9	66.9	81.5	98.1	d
>40-≤50		89.5	70.7	82.8	102.0	d
>50		108.2	76.5	93.6	125.9	e
≤10	Calving interval, days	388.9	371.0	383.0	400.0	a
>10-≤20		397.5	376.0	391.0	411.0	b
>20-≤30		408.4	382.0	401.0	424.0	c
>30-≤40		418.6	389.0	409.0	438.0	d
>40-≤50		425.6	391.0	416.0	448.5	d
>50		447.1	404.0	433.0	476.0	e

<sup>1</sup>Values lacking common superscript letters are significantly different ( $P < 0.05$ ) determined by the Dwass, Steel, Critchlow-Fligner multiple comparison procedure.

## Conclusion

Milk is the most promising matrix to identify cows with ketosis, because it is easy to sample and allows a complete screening of the herd. Based on routinely available clinical ketosis diagnoses and MIR-predicted traits, a new ketosis risk indicator (KetoMIR) was developed to provide a new screening tool of the herd at milk recording. The ketosis risk was modeled for the first 120 days of lactation. Overall, an increased incidence of cows with a moderate or high ketosis risk on the farm indicates a problem and was associated with lower milk production, increased somatic cell count, longer time from calving to first service and prolonged calving interval. The use of milk MIR-predicted traits for herd-level ketosis screening could be an important factor in preventing ketosis to limit as much as possible the economic losses caused by this disease.

## Data availability statement

The data used in the current study are not publicly available due to privacy restrictions of the data provider and owner (LKV Austria). Requests to access these datasets should be directed to F-JA [Franz-Josef.Auer@lkv-austria.at](mailto:Franz-Josef.Auer@lkv-austria.at).

## Ethics statement

This study was an observational study of farms in their normal routines, and no interventions were conducted on any animals or humans for the purpose of this article. It thus did not require Institutional Animal Care and Use Committee or Institutional Review Board approval.

## Author contributions

AK: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. LD: Writing – original draft, Writing – review & editing. AW: Writing – original draft, Writing – review & editing. MM: Writing – original draft, Writing – review & editing, Data curation. F-JA: Writing – original draft, Writing – review & editing. CE-D: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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## Conflict of interest

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