Correlations between the different aspects of ketosis dynamics in dairy herds in the Benelux by using BHB-measurements in milk from the DeLaval Herd Navigator™.

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Abstract

BHB-concentrations is mostly used for detection hyperketonaemia. Most studies applied BHB-concentrations in blood and a few studies have used BHB-measurements in milk. Onset, duration and maximum BHB-concentrations are different aspects of ketosis dynamics. The aim of this study is to investigate how different parameters of ketosis dynamics correlate with each other and the second objective of this study is to investigate if the severity of ketosis can be reflected in an area under the curve of the repeated BHB-measurements in milk detected with the DeLaval Herd Navigator™. Within the Herd Navigator an inline sampler inside the AMS automatically takes a representative sample of 80 ml milk from each individual cow during the milking process and measures BHB-concentrations at least daily from 3 to 20 days in milk and between 21 and 60 days in milk at least once every 4 days. The investigated data, provided by Lattec, which included 48 herds working with the Herd Navigator in the Benelux in June 2020. Data of unique calving's between July 2018 and August 2019 were included. The final dataset included 130,247 BHB-measurements of 4030 unique calving's. The current study defined three aspects of ketosis dynamics: the onset of ketosis, the duration of ketosis and the maximum BHB-concentration. Descriptive statistics were performed of the different aspects of ketosis. Mean onset of ketosis was 18 DIM, 15 DIM and 12 DIM for cows with parity 1, 2 and 3+ respectively. Mean duration of ketosis was 21 days for cows with parity 1, 27 days parity 2 cows and 32 days for cows with parity 3+. The correlation between the onset of ketosis and the duration of ketosis was -0.546 (parity 2 cows), the correlation between the duration and maximum BHB-concentration was 0.460 (parity 2 cows). The area under the curve is calculated as a sum of all BHB-measurements between 3 and 60 DIM. The mean area under the curve was 3,67 for parity 1 cows, 4,14 for parity 2 cows and 4,63 for parity 3+ cows. The current study shows a strong correlation between the different aspects of ketosis dynamics. Therefore, the area under the curve, including all three aspects of ketosis dynamics, is an interesting possibility to reflect the severity of ketosis in dairy cows.

Introduction

In dairy farming, most cows experience a negative energy balance (NEB) during the transition period. This NEB occurs due to an increased energy and glucose demand at parturition and decreased dry matter intake (DMI). A decreased DMI both before parturition and in the first weeks after calving is observed compared to the energy demand for milk production (Raboisson et al., 2014). Glucose is a fundamentally important for the maintenance of normal body functions and milk production. Cows unable to adequately transition through this period start mobilizing high amounts of body fat and, as a result, often develop hyperketonemia (HK), an excessive elevation of ketone bodies in the blood (McArt, 2013). HK is defined as a surplus of circulating ketone bodies (acetone, acetoacetate and β-hydroxybutyrate (BHB)) in blood. Ketone bodies are also present in milk and urine (Duffield, 2000).

Cows with HK are at higher risk of diseases postpartum, such as displaced abomasum and metritis (Suthar et al., 2013). In addition, cows with HK have shown a decreased milk yield, feed intake and a deteriorated reproductive performance (Ospina et al., 2010). Around 30 to 50% of dairy cows develop metabolic diseases, including HK, during the first 30 days in milk (LeBlanc, 2010; Berge and Vertenten, 2014). Costs per HK case has been estimated between €130,- and €257,- (Mostert et al., 2018; Raboisson et al., 2015)), and average herd level costs of HK are estimated at €3,613 per year for a herd with 130 cows (Steeneveld et al., 2020).

A deep NEB due to decreased DMI has been described as a main risk factor to develop HK, however there are more risk factors identified which increase the possibility for a cow to develop HK (Tatone et al., 2017). Cow associated risk factors include parity, high body condition score (BCS) at calving, lameness, breed, sex of the calf, twinning, calving interval in previous lactation and dry period length. Herd-level associated risk factors include milking system, average 305 milk yield of the herd and calving pattern of the herd (Tatone et al., 2017). Herds with an automatic milking system (AMS) have an increased within-herd prevalence. AMS allow more frequent milking, which contributes to an increase in milk yield and potentially in an increase of NEB (Tatone et al., 2017; De Jong, 2020).

A wide range of thresholds and methods for detection of HK have been reported (Berge and Vertenten, 2014; Benedet et al., 2019; Duffield, 2000; Krogh, et al 2011). BHB is mostly used for detection of HK, because it is predominant and a stable circulating ketone body in body fluids of cattle. Most studies applied a BHB-concentration in blood of ≥ 1.2 mmol/L as threshold for HK detection. Only a few studies have used a BHB-concentration in milk to detect HK (Koeck, et al., 2014; Tatone et al., 2017; Raboisson et al., 2014). An advantage of using milk samples is that it is less time-consuming and less stressful for dairy cows, and it provides good opportunities to be implemented in routine procedures in dairy farms.

Only a few studies investigated ketosis dynamics (McArt et al., 2013; Tatone et al., 2017; Santschi et al., 2016), such as onset, duration, prevalence, cumulative incidence and

maximum BHB-concentration. Ketosis dynamics results are based on blood (Berge and Vertenten, 2014) and milk measurements (Benedet et al.,2019). For instance, McArt et al. (2013) found an incidence of HK from 3 to 16 DIM of 45.7% based on blood and Tatone et al. (2017) found a peak prevalence between 6 and 7 DIM based on milk measurements. In another study, however, a peak prevalence in the first week postpartum for primiparous cows between 13 and 15 DIM for second-parity cows, and between 11 and 14 DIM for third parity and older cows was observed based on milk measurements (Santschi, et al. 2016). Recently, DeLaval Herd Navigator™ (HN) data was used to investigate ketosis dynamics (De Jong, 2020). The HN measures BHB-concentration in milk at least daily from 3 to 20 DIM and at least once every four days from 21 to 60 DIM. The threshold for HK is set at a BHB-concentration in milk of 0.08 mmol/L. Results found included for instance cumulative incidence of ketosis (defined as the proportion of cows with at least one sample with a BHB-concentration ≥0.08 mmol/L between 3 and 60 DIM) of 60.8% for all cows, and a median onset of ketosis of 18 DIM for primiparous cows and 13 DIM for multiparous cows (De Jong, 2020).

To our knowledge, no studies described the association among the different parameters of ketosis dynamics based on BHB measurements in milk. Therefore, the first objective of this study is to investigate how different parameters of ketosis dynamics correlate with each other (duration, BHB levels and onset after calving).
The second objective is to investigate if the severity of ketosis can be reflected in an area under the curve (AUC) of BHB between 3 and 20 DIM, 21-60 DIM and 3-60 DIM. Therefore, data on repeated BHB milk samples generated by the HN of Benelux farms will be analyzed. This study can provide more insights in different aspects of ketosis dynamics and the possible correlation between ketosis dynamics variables and ketosis severity.

Materials and Methods

Herd Navigator

Within the HN, an inline sampler inside the AMS automatically takes a representative sample of 80 ml milk from each individual cow during the milking process. HN analyses this milk sample and measures the BHB-concentration. BHB-concentration in milk is measured at least daily from 3 to 20 DIM and between 21 and 60 DIM fresh cows are sampled at least once every 4 days. Depending on the probability of ketosis (based on previous BHB-concentration or sudden drop or increase in milk yield), the HN will increase sample frequency (DelPro. – DeLaval Herd NavigatorTM)

Data collection

The database for this study is provided by Lattec. Lattec develops and supplies on farm sensor technology and was founded in 2001 and owned by DeLaval Int. AB. Lattec represents many different competences united into the HN product.
Lattec provided data from all dairy herds (48 herds) working with the HN in the Benelux in June 2020 and included all cows that calved before August 2019. The provided data included: herd identification, cow identity (CowId), herd size, milk equipment, unique cow number, parity, date of calving and BHB concentration in milk between 3 and 60 DIM.

Data editing

First, 10 herds are excluded because of working with the HN for a shorter period than 3 years, and 3 herds are excluded because of milking with a conventional milking system. After exclusion of the 13 herds, the data included 143,593 BHB measurements of 4,697 cows of 35 herds.
Further data-editing included aggregating of all farm data in one database for both general data and BHB-measurements data. General data included cow information such as: CowId, parity, parity group, herd, calving date and unique cow/herd-number. Parity was categorized in 3 categories (parity group 1, 2 and 3+).
For every unique cow and parity combination the following variables were defined: all BHB-measurements from 3 to 60 DIM and establishment per cow of the minimum and maximum BHB-level, the total number of BHB-samples within DIM 3 to 20, 21- 60 DIM and within 3-60 DIM $\geq$ 0,08 mmol/l and the first and last DIM with a BHB-measurement. Data editing was performed by using RStudio (Version 1.3.959 © 2009-2019 RStudio, Inc).

The exclusion criteria (figure 1) were established to adapt the dataset to exclude cows with a calving date before 01-07-2018 or after 03-08-2019. Subsequently, records from cows with no BHB-measurements at all were excluded. Also records with less than 14 BHB samples within DIM 3-20 and records with less than 8 BHB samples within DIM 21-60 were excluded. Exclusion criteria were established to adapt the dataset to retain cows with their first BHB sample at DIM 3-5 and their last BHB sample at DIM 56-60. The final dataset included data of 130,247 BHB-measurements of 4,030 unique calving's. Figure 1 shows the number of cows excluded per exclusion criterium.

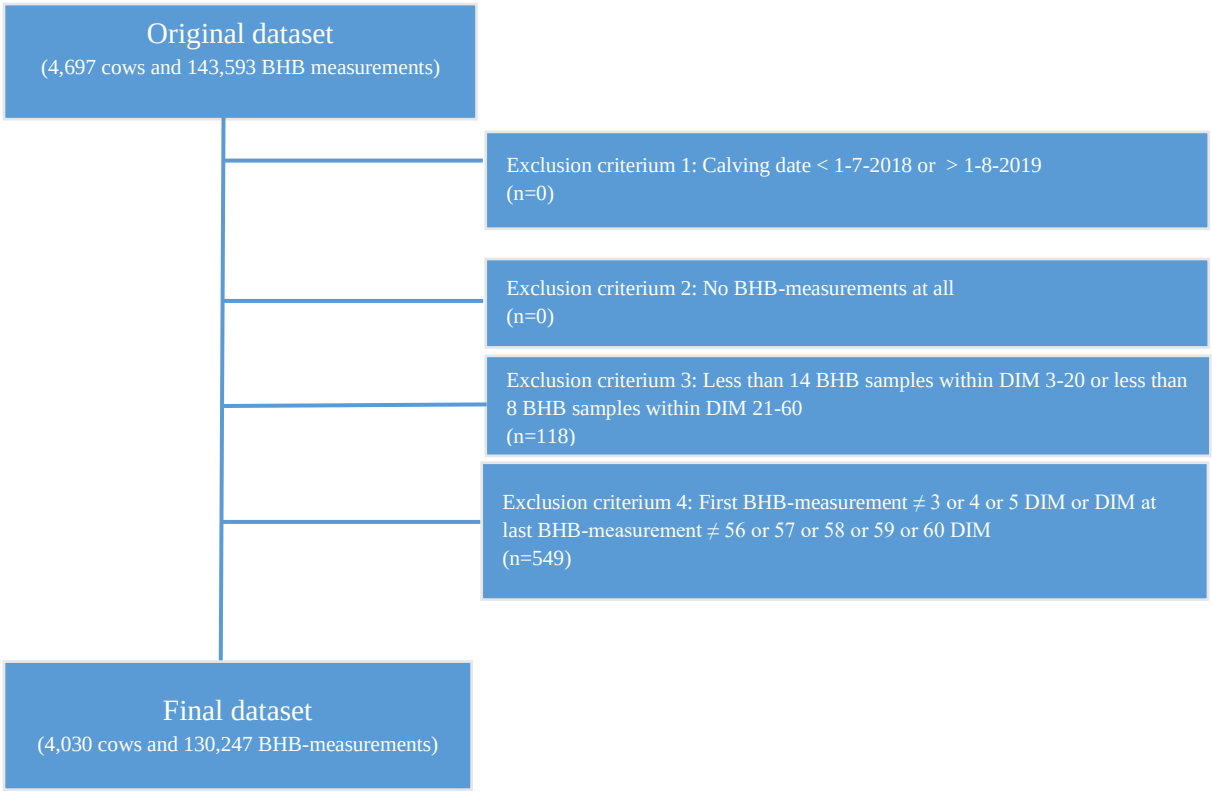

Figure 1: Exclusion criteria with number of observations.

To determine the AUC, for all DIM with missing BHB-measurement a BHB level was estimated as the mean of the nearest preceding DIM and nearest following DIM BHB-measurement. The AUC was calculated as the sum of all BHB-measurements for the following lactation segments: 3-20, 21-60 and 3-60 DIM.

After applying the exclusion criteria new variables were defined for all 4,030 unique calving's as follows:

- First day of BHB-measurement ≥ 0,08 mmol/l within 3-20 DIM
- Last day of BHB-measurement ≥ 0,08 mmol/l within 3-20 DIM
- Duration of BHB- measurement ≥ 0,08 mmol/l within 3-20 DIM

- First day of BHB-measurement ≥ 0,08 mmol/l within 21-60 DIM
- Last day of BHB-measurement ≥ 0,08 mmol/l within 21-60 DIM
- Duration of BHB- measurement ≥ 0,08 mmol/l within 21-60 DIM

- First day of BHB-measurement ≥ 0,08 mmol/l within 3-60 DIM
- Last day of BHB-measurement ≥ 0,08 mmol/l within 3-60 DIM
- Duration of BHB- measurement ≥ 0,08 mmol/l within 3-60 DIM

- AUC
- AUC within 3-20 DIM
- AUC within 21-60 DIM

- Maximum BHB-measurement within 3-20 DIM
- Maximum BHB-measurement within 21-60 DIM

Subsequently the ketosis dynamics parameters were defined (Table 1). A cow was considered positive for ketosis when a cow had at least one BHB-measurement with a BHB-concentration ≥ 0,08 mmol/l between 3-60 DIM. The duration of ketosis is subdivided into 3 segments: duration in days of BHB-measurements ≥ 0,08 mmol/l within 3-20 DIM, 21-60 DIM and 3-60 DIM.

Table 1: Definitions of the different ketosis dynamics parameters.

Ketosis dynamic parameter	Description
Onset of ketosis	The first DIM with a BHB-concentration ≥ 0,08 mmol/l.
Duration of ketosis	The number of subsequent days between the first and last DIM with a BHB-measurement ≥ 0,08 mmol/l.
Duration of ketosis 3-20 DIM	The number of subsequent days between the first and last DIM with a BHB-measurement ≥ 0,08 mmol/l within 3-20 DIM.
Duration of ketosis 21-60 DIM	The number of subsequent days between the first and last DIM with a BHB-measurement ≥ 0,08 mmol/l within 21-60 DIM.
Duration of ketosis 3-60 DIM	The number of subsequent days between the first and last DIM with a BHB-measurement ≥ 0,08 mmol/l within 3-60 DIM.
Maximum BHB-concentration	The highest BHB-concentration measured between 3-60 DIM.
Maximum BHB-concentration 3-20 DIM	The highest BHB-concentration measured between 3-20 DIM.
Maximum BHB-concentration 21-60 DIM	The highest BHB-concentration measured between 21-60 DIM.

Statistical analyses

Statistical analyses were performed with RStudio RStudio (Version 1.3.959 © 2009-2019 RStudio, Inc).

Descriptive statistics were performed for the number of cows that had a BHB-measurement ≥ 0.08 mmol/L and the prevalence of ketosis per parity group.

Subsequently, descriptive statistics were determined for ketosis dynamic parameters (onset, maximum and duration). Also, descriptive statistics were determined per parity group for the maximum BHB-measurements within 3-20 DIM, 21-60 DIM and 3-60 DIM.

Subsequently, correlations were determined between different parameters of ketosis dynamics (onset, duration and maximum BHB-measurements). The correlation was performed with the Pearson's product-moment correlation.

Descriptive statistics were determined for the AUC for all cows and per parity group (1, 2 and 3+). Subsequently, correlations found were determined between different parameters of

ketosis dynamics and the AUC (maximum BHB-concentration, onset, duration and number of days with a BHB-measurement ≥ 0,08 mmol/l).

Results

General herd information

Table 2 shows general information and herd characteristics of 4,030 cows of 35 different herds in the Benelux. In total 82.9% (n=29) of the herds were located in the Netherlands. Predominant breed was Holstein Friesian with 94.3% (n=33). The mean number of cows per herd was 115, with a mean proportion of cows with parity 1 of 28.6%. The categories parity 1, parity 2 and parity 3+ included 1,154, 1,033 and 1,843 cows, respectively.

Table 2: General information and herd characteristics of the 35 herds.

Variable	% (n)	Mean	Min	Max
Location				
Belgium	14.2 (5)			
Luxembourg	2.9 (1)			
The Netherlands	82.9 (29)			
Predominant breed				
Holstein Friesian	94.3 (33)			
Fleckvieh	5.7 (2)			
Year of Installation		2013	2010	2016
Herd Navigator™				
Number of cows		115.1	25	376
Proportion parity 1 (%)		28.6	6.0	38.6
Proportion parity 2 (%)		25.6	8.0	43.1
Proportion Parity 3+ (%)		45.7	28.0	71.4

Descriptive statistics

Table 3 shows characteristics of the dataset per parity group. Total number of cows that had a BHB-measurement ≥ 0.08 mmol/L is 3,018 cows (74.9). The percentage of animals in parity group 1 that had a BHB-measurement ≥ 0.08 mmol/L is 59.7% (n=689). Total percentage of animals in parity group 2 that had a BHB-measurement ≥ 0.08 mmol/L is 77.9% (n=805). Total percentage of animals in parity group 3+ that had a BHB-measurement ≥ 0.08 mmol/L is 83.1% (n=1,531).

Table 3: General characteristics of cows per parity group dataset of 35 herds.

	Parity group 1 mean (range)	Parity group 2 mean (range)	Parity group3 mean (range)
BHB-samples (days) *	31 (25-73)	32 (25-92)	34 (24-77)
Proportion first sample at 3 DIM (%)	99.05	99.81	99.99
Maximum BHB-concentration (mmol/L)	0.120 (0.053-0.985)	0.142 (0.055-0.898)	0.171 (0.047-0.966)
Mean days ≥ 0.08 mmol/L 3-60 DIM	7 (0-58)	9 (0-58)	13 (0-58)

*: Total number of days with a BHB-measurements within 3-60 DIM

Prevalence

The prevalence of ketosis per parity group was as followed: peak prevalence for cows with parity 1 was on 29 DIM, cows with parity 2 had a peak prevalence on 12 DIM and cows with parity 3+ had a peak prevalence on 14 DIM. Appendix 1-A1, A2, A3 show the prevalence per parity group after calving within 3-60 DIM.

Different aspects of ketosis dynamics

Onset of ketosis

Mean DIM for the onset of ketosis within 3-20 DIM for all cows was 8.0 DIM (range=3-20). The median was 7 DIM. The mean onset of ketosis per parity group can been seen in figure 2 and 3. Mean DIM for the onset of ketosis within 3-20 DIM for parity group 1 is 8.7, the mean DIM for parity group 2: 8.1 and for parity group 3+: 7.7 Mean DIM for the onset of ketosis within 3-60 DIM for all cows was 14.4 DIM (range=3-60) and the median was 9 DIM.

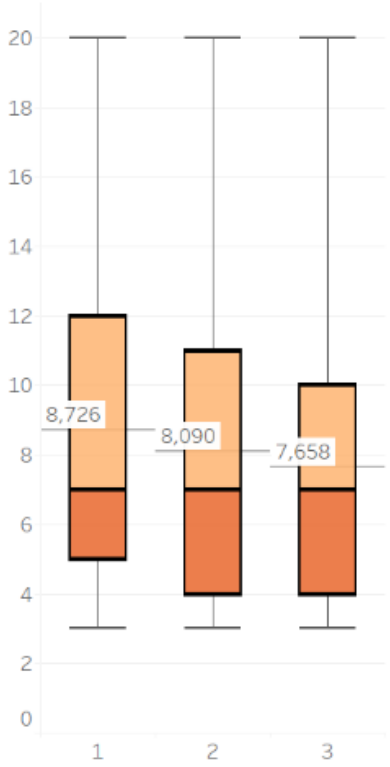

Figure 2: Boxplot and mean values of general onset of ketosis with BHB-measurements ≥ 0.08 mmol/l within 3-20 DIM per parity group (x-axis) and y-axis as DIM.

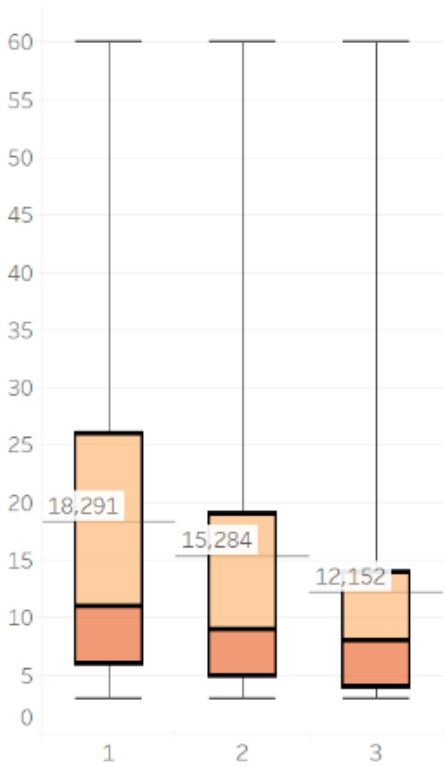

Figure 3: Boxplot and mean values of general onset of ketosis with BHB-measurements ≥ 0.08 mmol/l within 3-60 DIM per parity group (x-axis) and y-axis as DIM.

Duration of ketosis

In total 18.3% (n=553) of all cows which reach BHB-measurements ≥ 0.08 mmol/l has a duration of 1 day. A duration of only 1 day with ≥ 0.08 mmol/l BHB was measured in 30% (n=206), 20% (n=161) and 12% (n=186) of the cows from parity group 1, 2 and 3+ respectively

Mean duration (3-60 DIM) of ketosis for all cows was 28.3 days (range=1-58), with a median of 30 days. The mean duration (3-20 DIM) of ketosis for all cows was 8.6 days (range=1-18). Table 4 shows the duration of ketosis for the following lactation segments: 3-20, 21-60 and 3-60 DIM.

Table 4: Duration (in days) of ketosis for all cows for different lactation segments

	Duration 3-20 DIM	Duration 21-60 DIM	Duration 3-60 DIM
Minimum	1	1	1
1st Qu.	2	1	6
Median	9	19	30
Mean	8.6	18.4	28.3
3rd Qu.	14	33	48
Maximum	18	40	58

Figure 4 shows the duration (3-60 DIM) in days of ketosis per parity group.. Median duration of ketosis for cows in parity group 1 was 14 days, with lower and upper quartile of 1 and 41

days, respectively. Cows in parity group 2 had a median of 30 days with lower and upper quartile of 5 and 47 days, respectively. Cows in parity 3 or older had a median duration of 36 days with lower and upper quartile of 13 and 50 days, respectively.

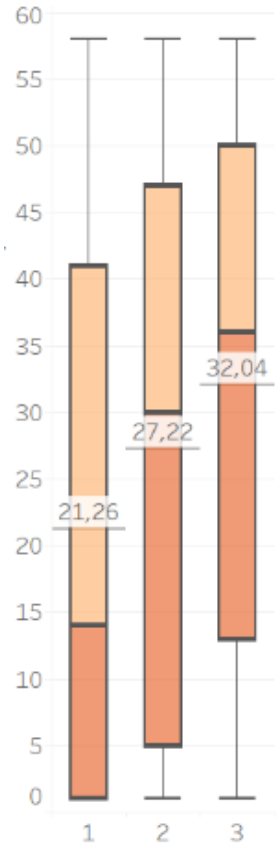

Figure 4: Boxplot of duration of ketosis per parity group (x-axis) and y-axis as days.

Maximum BHB-measurement

Mean maximum BHB-measurements of all cows was 0.149 mmol/l (range=0.047-0.985), with a median of 0.102 mmol/l. The mean maximum BHB-measurements per parity group can been seen in table 2. Mean maximum BHB-measurement within 3-20 DIM of all cows was 0.120 mmol/l (range= 0.049-0.985). Mean maximum BHB-measurement within 21-60 DIM of all cows was 0.130 mmol/l (range= 0.047-0.966). The mean maximum BHB-measurements within 3-20 DIM and 21-60 DIM per parity group can been seen in table 5.

Table 5: Mean maximum BHB-measurement (in mmol/l) per parity group

	Parity group 1	Parity group 2	Parity group 3+
Mean maximum BHB-measurement 3-20 DIM	0.097	0.117	0.137
Mean maximum BHB-measurement 21-60 DIM	0.107	0.125	0.148

Correlations between the different aspects of ketosis dynamics

Table 6, 7 and 8 shows the correlation per parity group for the onset of ketosis, maximum BHB-concentration, duration of ketosis and number of days with a BHB-measurement ≥ 0.08 mmol/l, within 3-60 DIM. The 95% CI lower and upper of those correlations can been seen in tables appendix 1-A4, A5 and A6.

Table 6: Correlations between ketosis dynamics parameters for parity 1 cows.

	Onset	Maximum	Duration 3-60	Number of days ≥ 0.08 mmol/L
Onset	1			
Maximum	-0.245	1		
Duration 3-60	-0.486	0.432	1	
Number of days ≥ 0.08 mmol/L	-0.368	0.797	0.631	1

Table 7: Correlations between ketosis dynamics parameters for parity 2 cows.

	Onset	Maximum	Duration 3-60	Number of days ≥ 0.08 mmol/L
Onset	1			
Maximum	-0.308	1		
Duration 3-60	-0.546	0.460	1	
Number of days ≥ 0.08 mmol/L	-0.416	0.792	0.630	1

Table 8: Correlations between ketosis dynamics parameters for parity 3 cows

	Onset	Maximum	Duration 3-60	Number of days ≥ 0.08 mmol/L
Onset	1			
Maximum	-0.287	1		
Duration 3-60	-0.516	0.448	1	
Number of days ≥ 0.08 mmol/L	-0.403	0.752	0.650	1

Area under the curve

Mean AUC 3-60 DIM for all cows was 4.37 (range = 2.04-27.99) and the median AUC was 3.64, with lower and upper quartile of 3.26 and 4.37, respectively. The mean AUC per parity group is shown in figure 5. The median AUC for parity group 1, 2 and 3+ is 3.33, 3.63 and 3.90, respectively. Figure 6 and 7 shows the AUC within 3-20 DIM and 21-60 DIM respectively. Figure 8 shows the correlations between the AUC and the different ketosis dynamic parameters.

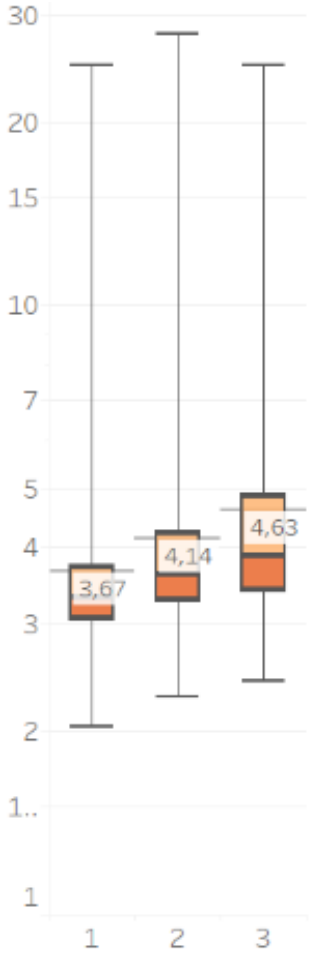

Figure 5: Boxplot of the AUC 3-60 DIM per parity group (x-axis), the mean is given as a number.

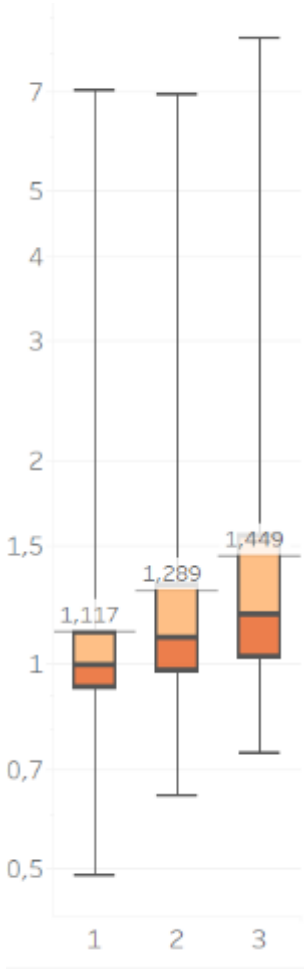

Figure 6: Boxplot of the AUC within 3-20 DIM per parity group (x-axis), the mean is given as a number.

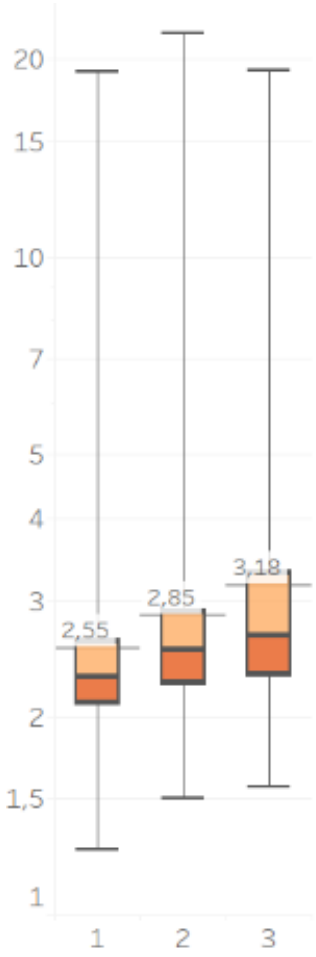

Figure 7: Boxplot of the AUC within 21-60 DIM per parity group(x-axis), the mean is given as a number.

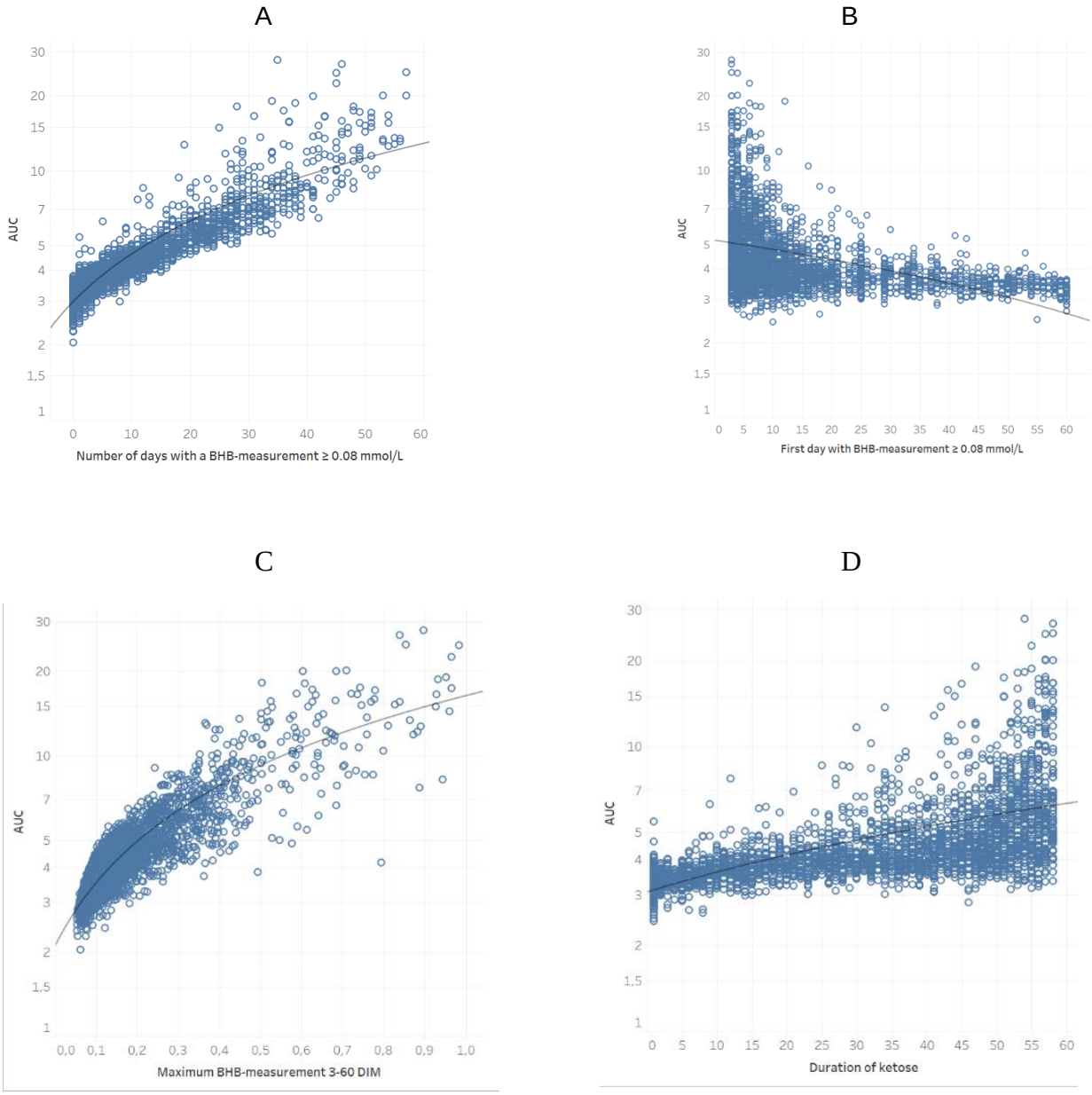

Figure 8: Four different aspects of ketosis dynamics (x-axis) and AUC (y-axis) for all cows (all y-axes are logarithmic).

Figure 8.a shows the mean number of days with a BHB-measurement ≥ 0.08 mmol/l and the AUC 3-60 DIM.
Figure 8.b shows the onset and the AUC 3-60 DIM
Figure 8.c shows the maximum BHB-measurements and the AUC 3-60 DIM
Figure 8.d shows the duration of ketosis and the AUC 3-60 DIM

Discussion

The current study determined how different parameters of ketosis dynamics correlate with each other and if the severity of ketosis can be reflected in an area under the curve. The results showed that there is a strong correlations between the different parameters of ketosis dynamics and strong correlations between the AUC and different parameters of ketosis dynamics. BHB-measurements with the HN were non-invasive, labour extensive and provided, due to the long sampling period and high sampling frequency, a unique insight into the different aspects of ketosis dynamics at cow-level.

Herds included in this study can be considered representative for Benelux farms in general concerning the predominant breed of the cows and the parity distribution. The average herd size of 115 cows is only slightly larger than average in the Netherlands (106) (Agrimatie, 2022) and larger than the 2020 average in Belgium (96) and Luxembourg (85) (LNV, 2020). Although herd size and parity distribution are representative, the included herds all use an automated milking systems (AMS). Within the Netherlands this type of milking parlour is used in 30,4% of the herds (Stichting KOM, 2022). Other research showed higher prevalence's of ketosis at farms with AMS (Tatone et al., 2017; De Jong, 2020). Furthermore, the herds included in the study differ from average herds due to the fact they work with a Herd Navigator™.

Onset of ketosis

Mean onset of ketosis was at 18 DIM for cows in parity group 1, 15 DIM for cows in parity group 2 and 12 DIM for cows in parity group 3+. This is similar to the study of De Jong (2020), who found mean onset of ketosis at 18 DIM for the primiparous and 13 DIM for the multiparous cows. Rathbun et al. (2017) found a mean onset of ketosis at 9 DIM. The study of Rathbun et al. (2017) used 4 samples between 5-18 DIM. The difference in mean onset of ketosis could be explained due to the long sample period in the current study. Rathbun et al. (2017) also used a threshold of 1.2 mmol/L but measured BHB in blood, while in the current study a threshold of 0.08 mmol/L in milk was used. McArt et al. (2012) found a mean and median onset of ketosis at 5 DIM. McArt et al. (2013) found a peak onset of ketosis at 5 DIM (diagnosed as a BHBA-concentration of ≥ 1.2 mmol/L in blood samples). However, in both studies (McArt et al. (2012) and McArt et al. (2013)) no distinction was made between parity groups.

Peak prevalence was at 29 DIM for cows in parity group 1, 12 DIM for cows in parity group 2 and 14 DIM for cows in parity group 3+. Tatone et al. (2017) found peak prevalence of ketosis using milk BHB tests at 6 and 7 DIM. In this study the threshold used was 0.15 mmol/L and no distinction between parity groups was made. Santschi et al. (2016) found a peak prevalence in the first week postpartum for primiparous cows, for second parity cows peak prevalence occurred between 13-15 DIM and for third parity and older cows peak prevalence was 11-14 DIM. Santschi et al. (2016) used the following thresholds to classify cows based on milk BHB-concentration: <0.15 mmol/L = negative, 0.15 - 0.19 is suspicious and ≥ 0.20 mmol/L = positive. This is different compared to the current study which considered a cow positive for ketosis when a cow had at least one BHB-measurement with a BHB-concentration ≥ 0,08 mmol/l.

Duration of ketosis

Mean duration of ketosis was 21.3 days for parity 1 cows, 27.2 days for cows with parity 2 and 32.0 days for parity 3+ cows. The median was 14, 30 and 36 days, respectively. De Jong (2020) found a duration of ketosis of 19.0 days for primiparous and 27.2 days for multiparous cows. In total 29.9% of parity 1 cows which reach a BHB-measurements ≥ 0.08 mmol/l had a duration of 1 day. 20% had a duration of 1 day for parity 2 cows and 15% had a duration of 1 day for parity 3+ cows. De Jong (2020) found slightly lower values for the percentage of duration of 1 day. Mahrt et al. (2015) found a median of duration of 3 to 4 days for BHB-measurements measured in blood, which is shorter than the current study and the study of De Jong (2020). Mahrt et al. (2015) tested twice a week for hyperketonaemia and defined the duration of ketosis as a duration from the first positive sample (blood BHB-measurement ≥1,2 mmol/l) till the first negative sample.

Maximum BHB-concentration

Mean maximum BHB-concentration for cows with parity 1 was 0.120 mmol/l, cows with parity 2 had a mean maximum BHB-concentration of 0.142 mmol/l and cows with parity 3+ had a mean maximum BHB-concentration of 0.171 mmol/l. The increasing mean maximum BHB-concentration with increasing parity is in agreement with other studies (Rathbun et al., 2017; De Jong, 2020).

Area under the curve

For all DIM without a BHB-measurement a BHB level was estimated. The fact that a DIM with missing BHB-measurement is filled with a calculated value is justified, especially because BHB is predominant and a stable circulating ketone body in body fluids of cattle. When a cow had a high BHB-measurement at 26 DIM for example, the same cow is likely to have a high BHB-measurement at 27 DIM (Hayirli et al., 2002).

However, the sampling pattern is based on a preset biomodel of the HN and not every cow is sampled every day. This is beneficial for the farmer from the perspective of avoiding costs of sampling. But this sampling pattern makes this data less standardized due to the bias in sampling.

A higher mean AUC found with higher parity (figure 5). No other studies have determined the AUC and the AUC is a possible new parameter duo to the fact that there is a strong correlation with the different aspects of ketosis dynamics and between the different aspects of ketosis dynamics and AUC. However, which parameter of ketosis dynamics (onset, duration, maximum BHB-concentration) contributes most to the AUC has not been investigated due to several reasons.

First of all, data on cow information has missing, such as; body condition score at calving, energy uptake during dry period, calving interval and dry period length. A high body condition score (≥3,5) at calving increases the risk of HK due to a lower DMI postpartum, resulting in a greater NEB (Vanholder et al., 2018; McArt et al., 2013; De Jong, 2020). Tatone et al. (2017) show an increasing odds of HK with longer calving interval, increased dry period length for multiparous cows and higher age at first calving for primiparous cows.

Secondly, herd level information was missing in the data, such as; average herd 305-day milk yield and calving pattern of the herd (McArt et al., 2012: Santschi et al., 2016: De Jong, 2020). A higher average herd 305-day milk yield increases the odds for HK (Vanholder et al., 2015; Tatone et al., 2017). Vanholder et al. (2015) show an association between the risk of HK and the quarter of the year of calving. For example, this information is needed to build a multivariable regression model. The multivariable regression model can provide more insights which parameters of ketosis dynamics contribute most to the AUC.

Recommendation for further research

A recommendation for further research is to investigate which factors contribute to the AUC level. Furthermore to investigate the consequences of differences in ketosis dynamics on health and performance in production and reproduction. Studies described a relation between cow performance and HK (McArt et al., 2012; Ospina et al., 2010), but these studies were using BHB-concentration measured in blood. The Herd Navigator™ also have other biomodels (reproduction and mastitis) with which research can be done in relation to health and performance in production and reproduction.

Conclusion

The mean onset of ketosis, defined as the first day with BHB-measurement ≥0.08 mmol/l within 3-60 DIM, was 18 DIM for cows with parity 1, 15 DIM for cows with parity 2 and 12 DIM for cows with parity 3+. Mean duration of ketosis was 21, 27 and 32 days for cows with parity 1, 2 and 3+ respectively, with increasing duration of ketosis for a higher parity. Mean maximum BHB-concentration is 0.120, 0.142 and 0.171 mmol/l for cows with parity 1, 2 and 3+ respectively. The correlation between the onset of ketosis and the duration of ketosis was -0.486 for cows with parity 1. -0.546 for cows with parity 2 and -0.516 for cows with parity 3+. The correlation between the maximum BHB-concentration and the duration of ketosis was 0.432, 0.460 and 0.448 for cows with parity 1, 2 and 3+ respectively. The correlation between the different aspects of ketosis suggest that a new parameter of ketosis dynamics, AUC, can be used as a new method of displaying the severity of ketosis. The mean AUC was 3,67 for cows with parity 1, cows with parity 2 had a mean AUC of 4,14 and cows with parity 3+ had a mean AUC of 4,63.

The current study shows that the different aspects of ketosis dynamics are strongly correlated between each other and the AUC is a possible candidate to reflect the severity of ketosis.

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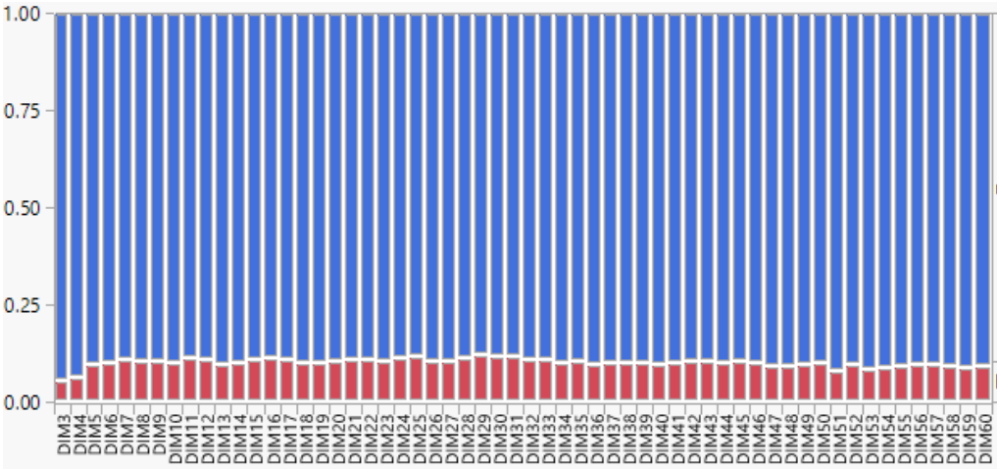
Appendix 1

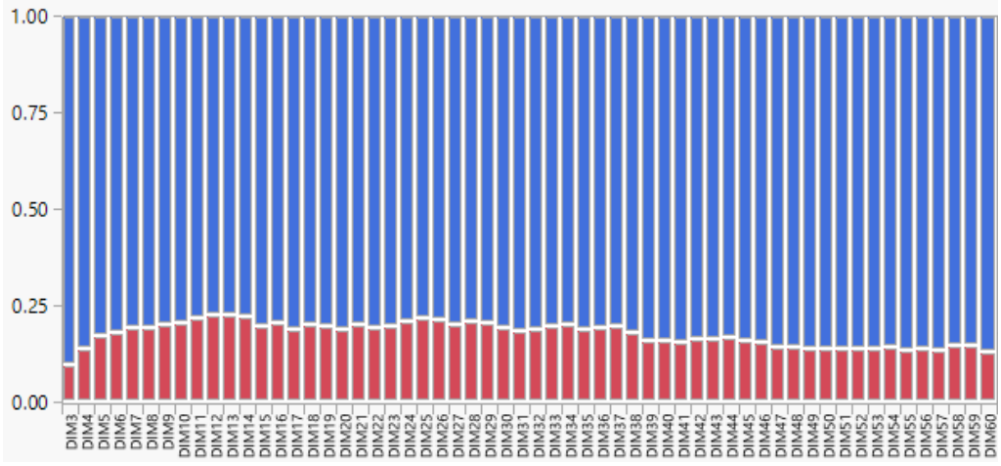
A1:

Prevalence for cows with parity 1. In this figure we see on the x-axis the DIM and on the Y-axis the prevalence proportion. Red the prevalence for Hyperketonaemia and blue the non HK. The highest prevalence is with 29 DIM



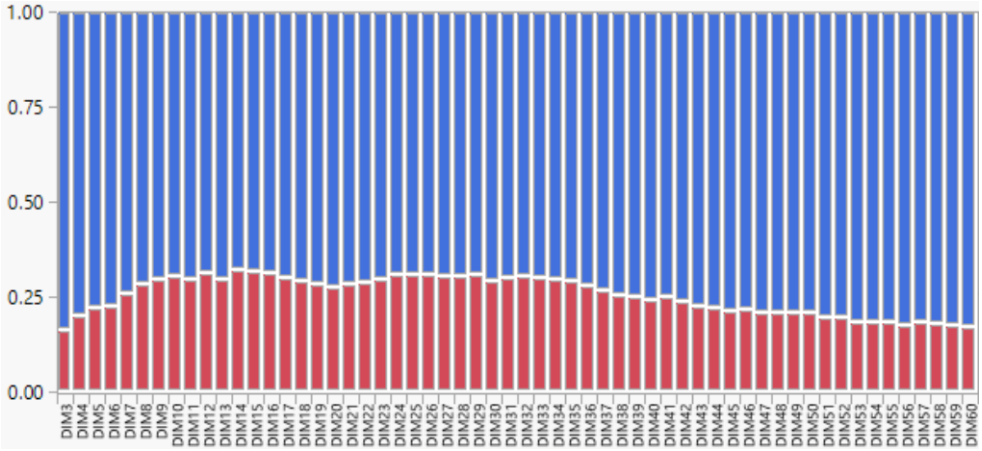
A2:

Prevalence for cows with parity 2. This figure shows the prevalence for cows with parity 2. The prevalence is higher than cows with parity 1. The highest prevalence is with 12 DIM



A3:

Prevalence for cows with parity 3+. This figure shows the prevalence for cows with parity 3+. The highest prevalence is with 14 DIM. But we see also a high prevalence in the fourth week after calving.



A4: Correlations for cows with parity 1.

Correlations between the onset of ketosis and other variables for cows with parity 1.

	Correlation	95% CI Lower	Upper
Maximum BHB-measurement (3-60)	-0.245	-0.314	-0.173
Duration 3-60 DIM	-0.486	-0.541	-0.427
Number of days with a BHB-measurement ≥ 0.08 mmol/L (3-60)	-0.368	-0.431	-0.301

Correlations between the duration of ketosis and other variables for cows with parity 1.

	Correlation	95% CI Lower	Upper
Maximum BHB-measurement	0.432	0.369	0.491
Number of days with a BHB-measurement ≥ 0.08 mmol/l.	0.631	0.577	0.669

Correlations between the maximum BHB-concentration and the number of days with a BHB-measurements ≥ 0.08 mmol/l for cows with parity 1.

	Correlation	95% CI Lower	Upper
Number of days with a BHB-measurement ≥ 0.08 mmol/l.	0.797	0.762	0.827

A5: Correlations for cows with parity 2

Correlations between the onset of ketosis and other variables for cows with parity 2.

	Correlation	95% CI Lower	Upper
Maximum BHB-measurement (3-60)	-0.308	-0.369	-00244
Duration 3-60 DIM	-0.546	-0.592	-0.495
Number of days with a BHB-measurement ≥ 0.08 mmol/L (3-60)	-0.416	-0.470	-0.355

Correlations between the duration of ketosis and other variables for cows with parity 2.

	Correlation	95% CI Lower	Upper
Maximum BHB-measurement	0.460	0.404	0.513
Number of days with a BHB-measurement ≥ 0.08 mmol/l.	0.630	0.587	0.670

Correlations between the maximum BHB-concentration and the number of days with a BHB-measurements ≥ 0.08 mmol/l for cows with parity 2.

	Correlation	95% CI Lower	Upper
Number of days with a BHB-measurement ≥ 0.08 mmol/l.	0.792	0.765	0.817

A6: Correlations for cows with parity 3+

Correlations between the onset of ketosis and other variables for cows with parity 3+.

	Correlation	95% CI Lower	Upper
Maximum BHB-measurement (3-60)	-0.287	-0.332	-0.240
Duration 3-60 DIM	-0.516	-0.552	-0.478
Number of days with a BHB-measurement ≥ 0.08 mmol/L (3-60)	-0.403	-0.444	-0.360

Correlations between the duration of ketosis and other variables for cows with parity 3+.

	Correlation	95% CI Lower	Upper
Maximum BHB-measurement	0.448	0.407	0.487
Number of days with a BHB-measurement ≥ 0.08 mmol/l.	0.650	0.620	0.678

Correlations between the maximum BHB-concentration and the number of days with a BHB-measurements ≥ 0.08 mmol/l for cows with parity 3+.

	Correlation	95% CI Lower	Upper
Number of days with a BHB-measurement ≥ 0.08 mmol/l.	0.752	0.730	0.773